March 23, 2016

Mr. Rick Yarde, Regional Supervisor
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Bureau of Ocean Energy Management
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Camarillo, CA 93010

Mr. David Fish, Acting Chief
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1849 C Street, N.W., Room 5429
Washington, D.C. 20240

Sent via email to pocswellstim@anl.gov

Re: Comments on the Draft PEA for Well Stimulation Treatments on the Pacific OCS

Dear Mr. Yarde and Mr. Fish:

These comments on the Bureau of Ocean Energy Management's ("BOEM") and Bureau of Safety and Environmental Enforcement's ("BSEE") draft programmatic environmental assessment ("draft PEA") for well stimulation treatments ("WST") on the Southern California Outer Continental Shelf are submitted on behalf of the Environmental Defense Center ("EDC") and the Surfrider Foundation. The Southern California Outer Continental Shelf currently contains 43 active leases and 22 production platforms, 19 located within the Santa Barbara Channel, and three located offshore Long Beach and northern Orange County.

EDC is a nonprofit environmental law firm that protects and enhances the environment through education, advocacy and legal action. EDC represents itself and other organizations on work related to coast and ocean resources, the Santa Barbara Channel, clean water, open spaces and wildlife, and climate and energy.

The Surfrider Foundation ("Surfrider") is a non-profit grassroots organization dedicated to the protection and enjoyment of our world's oceans, waves and beaches. Surfrider now maintains over 90 chapters worldwide and is fueled by a powerful network of activists.

EDC and Surfrider have reviewed the Draft PEA with the assistance of Blue Tomorrow, LLC ("Blue Tomorrow"), an environmental consulting company that

specializes in assessing environmental impacts from oil and gas operations. This expert consultant has prepared written comments that are attached hereto, and which are incorporated in their entirety. Please ensure that you separately respond to Blue Tomorrow's expert comment letter.

Summary

EDC and Surfrider Foundation appreciate the opportunity to comment on the draft PEA. Our organizations disagree with the draft PEA conclusion, however, that offshore fracking and acidizing from the 22 southern California offshore oil platforms will have no environmental impact. In addition, as we detail in this letter, the draft PEA is legally insufficient under the National Environmental Policy Act ("NEPA") in numerous respects. We strongly encourage BOEM and BSEE to initiate preparation of an Environmental Impact Statement ("EIS") that acknowledges the significant environmental impacts and risks associated with offshore fracking and acidizing, and that provides a more detailed and thorough analysis of those impacts and risks.

Background

The U.S. Department of the Interior ("DOI") recently defined hydraulic fracturing (*aka* 'fracking') as:

> involv[ing] the injection of fluid under high pressure to create or enlarge fractures in the reservoir rocks. The fluid that is used in hydraulic fracturing is usually accompanied by proppants, such as particles of sand, which are carried into the newly fractured rock and help keep the fractures open once the fracturing operation is completed. The proppant-filled fractures become conduits for fluid migration from the reservoir rock to the wellbore and the fluid is subsequently brought to the surface. In addition to the water and sand (which together typically make up 98 to 99 percent of the materials pumped into a well during a fracturing operation), chemical additives are also frequently used. These chemicals can serve many functions in hydraulic fracturing, including limiting the growth of bacteria and preventing corrosion of the well casing. The exact formulation of the chemicals used varies depending on the rock formations, the well, and the requirements of the operator.

Department of the Interior, Bureau of Land Management Final Rule: Oil and Gas; Hydraulic Fracturing on Federal and Indian Lands, 80 Fed. Reg. 16,128 (Mar. 26, 2015).

In contrast to fracking, acidizing uses the application of one or more acids, typically hydrofluoric acid and hydrochloric acid, to the well or underground geologic

formation. Reflecting its prevalent use in the state, California became the first state to directly regulate acidizing, as well as fracking, with the enactment of Senate Bill 4 ("SB 4") in 2014. Under that law, "acid well stimulation treatment" is defined as "the application of one or more acids to the well or underground geologic formation," which "may be at any applied pressure and may be used in combination with hydraulic fracturing treatments or other well stimulation treatments." Cal. Pub. Res. Code § 3158 (2014). According to the American Petroleum Institute, acidizing has been used to improve well productivity for many years, and is "one of the most widely used and effective means available for improving the productivity (stimulation) of wells."[1]

In its Findings for SB 4, the California Legislature in 2014 declared that "[i]nsufficient information is available to fully assess the science of the practice of . . . well stimulation treatment technologies in California, including environmental, occupational, and public health hazards and risks," and accordingly, that "[p]roviding transparency and accountability to the public regarding well stimulation treatments . . . is of paramount concern." Section 1(b),(c). Last year, an independent scientific study addressing the environmental and public health impacts of fracking and acidizing prepared by the nonpartisan California Council on Science and Technology ("CCST") pursuant to that law reached a similar conclusion, with the study's authors stating that "only incomplete information and data exist," and that "[f]ew scientific studies of the health and environmental impacts of well stimulation have been conducted to date, and the ones that have been done focus on other parts of the country." CCST, *An Independent Scientific Assessment of Well Stimulation in California: Volume II. Potential Environmental Impacts of Hydraulic Fracturing and Acid Stimulations* at 6 (July 2015). The numerous gaps in information include the "concentration of well stimulation chemicals, their degradation products, and natural constituents mobilized" by fracking and acidizing. *Id*. at 336.

The deficiency of information concerning offshore fracking and acidizing is even more pronounced than their onshore use.[2] Unlike onshore fracking, DOI has not initiated

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[1] API Briefing Paper: Acidizing Treatment in Oil and Gas Operations. Available at: http://www.api.org/~/media/files/oil-and-natural-gas/hydraulic-fracturing/acidizing-oil-natural-gas-briefing-paper-v2.pdf.

[2] Indeed, the use of fracking and acidizing off California's shores was largely unknown to the general public, local elected officials, and cooperating state agencies until less than two years ago, when investigative reporters, and EDC, discovered its use through records obtained under the Freedom of Information Act ("FOIA"), 5 U.S.C. § 552; EDC published its analysis in a report entitled DIRTY WATER: FRACKING OFFSHORE CALIFORNIA, along with policy recommendations directed at BOEM, BSEE, and DOI, including to stop relying on categorical exclusions to approve well stimulation methods, until they have thoroughly studied the impacts and provided for public participation.

a rulemaking or other public process to address the use of offshore fracking and other well stimulation techniques, nor provided the public with any estimates of the prevalence of well stimulation, or the extent of its expected use in the future.

This lack of prior consideration or analysis of offshore fracking and acidizing, combined with a complete lack of transparency into BOEM and BSEE's approval of permits authorizing these activities, led EDC to file a federal lawsuit against the agencies in late 2014 alleging numerous violations of NEPA.

EDC's lawsuit challenged BOEM and BSEE's failure to provide for any public participation or conduct adequate environmental review in connection with the approval of fifty-one specific Applications for Permits to Drill ("APDs") and Applications for Permits to Modify ("APMs") authorizing WSTs. *Environmental Defense Center v. Bureau of Safety and Environmental Enforcement*, No. 2:14-cv-09281 (C.D. Cal. Dec. 3, 2014) (*EDC v. BSEE*). The majority of the challenged APDs and APMs approved acidizing rather than fracking. Specifically, EDC's action challenged BOEM and BSEE's 1) failure to provide for public participation as required by NEPA; 2) unlawful reliance on categorical exclusions despite evidence of significant and cumulative environmental effects; 3) unlawful reliance on categorical exclusions despite extraordinary circumstances; 4) unlawful reliance on categorical exclusions to approve APDs despite lack of applicability; 5) failure to conduct any NEPA analysis for APMs; and 6) unlawful reliance on categorical exclusions for APMs. Complaint at 32–39, *EDC v. BSEE* (No. 1).

In January 2016, EDC reached a settlement agreement with BOEM and BSEE that requires the agencies to prepare a Programmatic Environmental Assessment ("PEA") addressing environmental impacts of offshore well stimulation in federal waters off California, and issue the final environmental review document by May 28, 2016, after a public comment period of at least 30 days. Settlement Agreement, *EDC v. BSEE* (Settlement lodged Jan. 29, 2016, No. 79-1). The settlement requires the agencies to withhold approval of drilling permits authorizing well stimulation pending completion of the PEA. In addition, if the agencies determine during the PEA process that well stimulation may have significant environmental impacts, they must prepare a Programmatic Environmental Impact Statement ("PEIS"). Finally, under the settlement, BSEE must develop an electronic filing and public notification web site for offshore drilling permit applications, and post completed applications to the system within five days. *Id.*

Specific Comments

Purpose and Need

NEPA requires federal agencies proposing actions to "briefly specify the underlying purpose and need to which the agency is responding in proposing the alternatives including the proposed action." 40 C.F.R. §§ 1502.13, 1508(9)(b); 43 Fed. Reg. 45,983 (1979). The purpose and need statement "is an obvious place for the court to start when analyzing the adequacy of an environmental impact statement [or environmental assessment]," as "[i]t is from this statement that the agency, public, and ultimately, the court may begin to judge whether the agency has fully analyzed the possible impacts of the action and reviewed a reasonable range of alternatives to that action." *Soda Mountain Wilderness Council v. Norton*, 424 F. Supp. 2d 1241, 1261 (E.D. Cal. 2006).

In defining a purpose and need statement, an action agency must place particular weight on the relevant statutes and other authorities that define its legal duties and responsibilities in relation to the proposed project or program. *Citizens Against Burlington v. Busey*, 938 F. 2d 190, 196 (D.C. Cir. 1991) ("[A]n agency should always consider the views of Congress, expressed, to the extent that the agency can determine them, in the agency's statutory authorization to act, as well as in other congressional directives."). Moreover, the definition of a purpose and need statement under NEPA cannot be entirely driven by private party applicants, permittees, or lessees. *See Van Abbema v. Fornell*, 807 F. 2d 633, 638 (7th Cir. 1986) ("the evaluation of 'alternatives' mandated by NEPA is to be an evaluation of alternative means to accomplish the general goal of an action; it is not an evaluation of the alternative means by which a particular applicant can reach his goals."). Accordingly, a purpose and need statement that states a purpose to enact or adopt a private party applicant's proposal is unlawfully narrow. *National Parks & Conservation Ass'n v. Bureau of Land Management*, 606 F. 3d 1058, 1069-72 (9th Cir. 2010); *Theodore Roosevelt Conservation Partnership v. Salazar*, 661 F. 3d 66, 73 (D.C. Cir. 2011).

The draft PEA fails to meet these legal requirements by including a purpose and need statement that is driven entirely by the desire of oil company lessees to conduct offshore fracking and acidizing. Specifically, BOEM and BSEE incorrectly define the purpose and need statement as "to allow the use of certain WSTs (e.g. hydraulic fracturing) in support of oil production at platforms on the Pacific OCS." Draft PEA at ES-1.

The legal settlement entered into by EDC with BOEM and BSEE that compelled this programmatic environmental analysis further undermines the notion that the purpose

of the PEA is simply to facilitate offshore WST practices. Settlement Agreement, *EDC v. BSEE* (No. 79-1). As stated in the settlement, the PEA is a central obligation agreed to by the agencies in order to resolve our numerous alleged claims that the agencies have routinely violated NEPA in their issuances of categorical exclusions for offshore fracking and acidizing. Under the settlement, the purpose of the PEA is for the agencies, *for the first time*, to consider the potential environmental impacts of offshore well stimulation, and then based on that analysis, determine whether further offshore well stimulation should be permitted or otherwise authorized. Indeed, BOEM and BSEE agreed that they "will not pre-determine the outcome of this assessment." The purpose and need statement runs directly afoul of this binding settlement commitment, as well as NEPA's underlying requirements, by not only presuming that offshore fracking and acidizing can be done safely and in conformance with governing laws, but that BOEM and BSEE have an obligation to promote their use.

Moreover, the overly narrow purpose and need statement does not reflect or acknowledge the requirements of the Outer Continental Shelf Lands Act ("OCSLA"), 43 U.S.C. §§ 1331–1356b, requiring BOEM and BSEE to *balance* oil production with environmental protection, nor does it acknowledge numerous other applicable environmental laws such as the Endangered Species Act, Clean Water Act, and Coastal Zone Management Act. Originally enacted in 1953, the OCSLA reaffirmed federal control over resources on the Outer Continental Shelf ("OCS"), located beyond three nautical miles from a state's coast. OCSLA requires that oil exploration and production be "balanced with 'protection of the human, marine, and coastal environments.'" *Natural Resources Defense Council v. Hodel*, 865 F. 2d 288, 292 (D.C. Cir. 1988) (*quoting* 43 U.S.C. § 1802(1)–(2)). BOEM and BSEE cannot narrowly interpret OCSLA to limit the scope of their NEPA analysis. *See Ctr. for Biological Diversity v. Nat'l Highway Traffic Safety Admin.*, 538 F.3d 1172, 1213 (9th Cir. 2008) (*quoting Forelaws on Board v. Johnson*, 743 F.2d 677, 683 (9th Cir.1985)) ("NEPA's legislative history reflects Congress's concern that agencies might attempt to avoid any compliance with NEPA by narrowly construing other statutory directives to create a conflict with NEPA. Section 102(2) of NEPA therefore requires government agencies to comply 'to the fullest extent possible.'"). Although the purpose and need statement does acknowledge that BOEM and BSEE must comply with the OCSLA, the presumption that the agencies shall allow the use of offshore fracking and acidizing puts the cart before the horse, and calls the overall objectivity of the draft PEA into question.

Project Alternatives

Using the purpose and need statement as a foundation, federal agencies are directed under NEPA to "study, develop, and describe alternatives to recommended courses of action in any proposal which involves unresolved conflicts concerning

alternative uses of available resources. . . .” 42 U.S.C. § 4332(2)(E). The discussion of alternatives is “the heart” of the NEPA process, and is intended to provide a “clear basis for choice among options by the decisionmaker and the public.” 40 C.F.R. § 1502.14; *Idaho Sporting Congress v. Alexander*, 222 F. 3d 562, 567 (9th Cir. 2000) (compliance with NEPA’s procedures “is not an end in itself . . . [but] it is through NEPA’s action forcing procedures that the sweeping policy goals announced in § 101 of NEPA are realized.”). As purpose and need statements are one of the main engines driving the alternatives analysis within a NEPA document, failure to properly define a project’s purpose and need will in turn preclude proper consideration of a reasonable range of alternatives. *National Parks Conservation Ass’n*, 606 F. 3d at 1072 (“As a result of this unreasonably narrow purpose and need statement, the BLM necessarily considered an unreasonably narrow range of alternatives.”).

Like an agency’s determination of a project’s purpose and need, the range of alternatives may not be entirely driven by a private applicant’s preferences. *See* Forty Most Asked Questions Concerning CEQ’s NEPA Regulations, 48 Fed. Reg. 18,026 (March 16, 1981) (“In determining the scope of alternatives to be considered, the emphasis is on what is ‘reasonable’ rather than on whether the proponent or applicant likes or is itself capable of carrying out the particular alternative. Reasonable alternatives include those that are practical or feasible from a technical and economic standpoint and using common sense, rather than simply desirable from the standpoint of the applicant.”). The agencies must “rigorously explore and objectively evaluate all reasonable alternatives.” 40 C.F.R. § 1502.14.

In this case, BOEM and BSEE impermissibly narrowed the scope of the purpose and need statement, and in turn unlawfully constrained their consideration of alternatives and rendered the draft PEA an empty formality. Although the agencies developed two alternatives that would place some restrictions on the use of offshore fracking and acidizing, by prohibiting the use of fracturing WSTs at depths less than 2,000 feet and prohibiting open water discharge of WST waste fluids, respectively, the agencies inexplicably failed to consider the restrictions together in one alternative, or to otherwise craft a comprehensive alternative that would best preserve the environment in the event that future WST treatments are allowed by the agencies. *Oregon Natural Desert Association v. Bureau of Land Management*, 531 F. 3d 1114 (9th Cir. 2008); *Natural Resources Defense Council v. U.S. Forest Service*, 421 F. 3d 79 (9th Cir. 2005). Additional alternatives that further restrict WSTs would be reasonably related to the project’s proper purpose, which should be whether offshore WST can safely occur, in light of OCSLA’s requirement to balance resource extraction with environmental protection. *See Ctr. for Biological Diversity*, 538 at 1219 (concluding the agency failed to analyze a reasonable range of alternatives, and that a more environmentally protective

alternative was reasonably related to the project's purpose that included energy conservation).

Additionally, and as discussed in more detail below, BOEM and BSEE gave short shrift to the alternatives considered due to their unsupported conclusions that offshore fracking and acidizing will essentially cause no environmental impacts. *See* Draft PEA at 4-60 ("Under Alternative 1, the use of any of the four WSTs included in the alternative is expected to have at most only limited or negligible impacts on potentially affected resources."); *id*. at 4-71 ("In conclusion, neither the proposed action nor any of the action alternatives are expected to result in more than short-term, localized impacts on the environment."). This overarching deficiency poisons the adequacy of the entire analysis—in order to take the required "hard look" at a proposed project's effects as required by NEPA, an agency may not rely on incorrect assumptions or data. 40 C.F.R. § 1500.1(b). It also undermines the adequacy of the alternatives analysis as in essence, BOEM and BSEE have concluded that they need not give any credence to alternatives that would in any manner constrain, condition, or mitigate the impacts of offshore fracking and acidizing based on their arbitrary and capricious assertions that those practices have no impacts to the environment. In relying on inaccurate and unsupported data, the draft PEA runs afoul of NEPA's mandate that the agencies must "[r]igorously explore and objectively evaluate all reasonable alternatives." *Sierra Forest Legacy v. Rey*, 577 F. 3d 1015, 1022 (9th Cir. 2009) (*citing* 40 C.F.R. § 1502.14).

Incomplete or Unavailable Information

NEPA's implementing regulations place specific obligations on agencies considering a proposed action with incomplete or unavailable information. Under those regulations, when there is incomplete or unavailable information regarding potential environmental impacts, the agency shall always make clear that such information is lacking. 40 C.F.R. § 1502.22. As stated by the Ninth Circuit, "general statements about 'possible effects' and 'some risk' do not constitute a 'hard look' absent a justification regarding why more definitive information could not be provided." *Blue Mountains Biodiversity Project v. Blackwood*, 161 F.3d 1208, 1213 (9th Cir. 1998) (internal citations omitted). Instead, an "agency must generally prepare an EIS if the environmental effects of a proposed agency action are highly uncertain . . . [and] where uncertainty may be resolved by further collection of data, or where the collection of data may prevent speculation on potential effects." *National Parks*, 241 F. 3d at 731; *see also Sierra Club v. United States Forest Serv*., 843 F. 2d 1190, 1195 (9th Cir. 1988) ("The purpose of an EIS is to obviate the need for speculation by insuring that available data are gathered and analyzed prior to the implementation of the proposed action.").

In this instance, the draft PEA suffers from missing information and numerous data gaps, many pertaining to the most concerning and contentious aspects of offshore well stimulation, including the toxicity of chemicals utilized in the process, as well as the impact of those chemicals on the natural environment, including water quality, threatened and endangered species, and human health.

Illustrating these significant gaps in knowledge, the CCST Study cited extensively throughout the draft PEA used the word "unknown" 87 times in Volume II, which addresses potential environmental impacts. The study further notes that as many as 100 chemicals used in WST have "completely unknown materials." CCST, Volume II at p. 81. Other fundamental information gaps noted in the study include the amount of frack fluid that returns to the surface and how much remains underground. In addition, as the CCST Study notes, "discharges are not monitored for constituents specific to or indicative of hydraulic fracturing, and the timing of sampling is unlikely to coincide with or measure any potential impacts from well stimulation treatments." *Id*. at p. 103.

The lack of study and information in relation to acidizing techniques is even more pronounced than in relation to fracking. As noted in a recently issued study of acidizing in California, "[w]hile researchers have begun exploring the potential impacts of hydraulic fracturing more seriously, impacts from acidizing are not being examined as closely. It is important that acidizing be a bigger part of the discussion to protect the public and environment from potential harm." Khadeeja Abdullah, Timothy Malloy, Michael K. Stenstrom & I.H. Suffet (2016): *Toxicity of acidization fluids used in California oil exploration*, Toxicology & Environmental Chemistry.[3]

As further discussed in detail in that study, there are close to 200 specific chemicals used in acidizing, with at least 28 of those chemicals being "F-graded" (known carcinogens, mutagens, reproductive toxins, developmental toxins, endocrine disrupters, or high acute toxicity chemicals), including hydrofluoric acid, xylene, diethylene glycol, and ethyl benzene. Moreover, almost 90 additional chemicals used in the acidizing process cannot even be identified by a specific name, due to trade secret protections. These enormous gaps in knowledge are compounded by the fact that acidizing relies on chemical concentrations that greatly exceed those used in fracking (6-18% vs. 0.5 %), and relies heavily on hydrofluoric acid, which has "very high acute mammalian toxicity and neurotoxicity."

In addition, Blue Tomorrow identified specific data gaps in the draft PEA that render a realistic assessment of impacts impossible without more data and analysis.

[3] Available online at: (http://dx.doi.org/10.1080/02772248.2016.1160285). This study is attached and should be added to the administrative record for the PEA process and decision.

Specifically, the draft PEA's discharge toxicity analysis is inadequate because it contains a significant data gap regarding the composition of flowback fluids. Blue Tomorrow Expert Letter at 1 ("During WSTs additional constituents are being mobilized from the formation and their chemistry and toxicity are unknown. Quantifying the risk from discharging these fluids is not possible without this information."). In addition, the draft PEA completely lacks direct evidence on the impacts of discharges of WST flowback fluids on the marine environment. *Id.* at 2 ("As a result of the absence of scientific studies of impacts to the marine environment from WST waste discharges, the EA evaluation is insufficient to support the conclusion that no WST-related impacts to ecological resources are expected to occur."). Moreover, "only a fraction of chemicals had toxicity data for marine organisms (26 of the 33 chemicals screened in the hydraulic fracturing fluid; and 5 of the 17 chemicals screened in the acidizing case study)." *Id.* at 3. The draft PEA fails to adequately acknowledge these numerous and fundamental data gaps and missing information, and consequent uncertainty regarding environmental impacts. In any event, even if acknowledged, these gaps are so significant to compel preparation of an EIS.

Direct Environmental Effects

As noted above, under the *Environmental Defense Center v. Bureau of Safety and Environmental Enforcement*, Case No. 2:14-cv-9281 (C.D. Cal.) settlement agreement, BOEM and BSEE agreed to prepare a PEA. The agreement further specifies that the PEA "will result in a determination that either an [EIS] and Record of Decision (ROD) is required or a Finding of No Significant Impact (FONSI) is appropriate," and further, that the agencies "will not pre-determine the outcome of this assessment to require one product or the other before the analysis in the programmatic EA is complete."

This settlement language conforms with NEPA and its implementing regulations. When a federal agency is not certain whether an EIS is required, it must prepare an environmental assessment ("EA"). 40 C.F.R. § 1508.9. If the agency concludes in an EA that a project may have significant impacts on the environment, then an EIS must be prepared. 40 C.F.R. § 1501.4. If an EA concludes that there are no significant impacts to the environment, the federal agency must provide a detailed statement of reasons why the project's impacts are insignificant and issue a "finding of no significant impact" ("FONSI"). 40 C.F.R § 1508.13.

In determining whether a proposed action may significantly affect the environment, NEPA requires that both the context and intensity of that action be considered. 40 C.F.R. § 1508.27. In considering context, "[s]ignificance varies with the setting of the proposed action." *Id.* § 1508.27 (a). Consideration of intensity, on the other hand, "refers to the severity of the impact," including impacts on "[u]nique

characteristics of the geographic area such as proximity to park lands . . . wetlands . . . or ecologically critical areas," "[t]he degree to which the action may establish a precedent for future actions with significant effects or represents a decision in principle about a future consideration," and "[w]hether the action is related to other actions with individually insignificant but cumulatively significant impacts." *Id.* § 1508.27(b). The courts have repeatedly emphasized that "[t]he presence of one such factor may be sufficient to deem the action significant." *Nat'l Parks & Conservation Ass'n v. Babbitt*, 241 F. 3d 722, 731 (9th Cir. 2001); *Ocean Advocates v. U.S. Army Corps of Engineers*, 402 F. 3d 846, 864–65 (9th Cir. 2005) (EA and FONSI inadequate when agency fails to prepare adequate cumulative impacts analysis).

In the draft PEA, BOEM and BSEE analyze the following categories of potential environmental impacts: air quality (including greenhouse gas emissions); water quality; geologic resources/seismicity; benthic resources; marine and coastal fish and essential fish habitat; marine and coastal birds; marine mammals; sea turtles; commercial and recreational fisheries; areas of special concern; recreation and tourism; environmental justice; and archeological resources. Draft PEA at ES-7. Almost without exception, BOEM and BSEE conclude that the proposed action Alternative 1 to allow use of offshore fracking and acidizing will result in "no WST-related impacts expected." Draft PEA at ES-11 and ES-12 (Table ES-1). Only with respect to water quality ("slight localized reduction in water quality at surface water discharge location"), induced seismicity ("low potential"), and marine fish and wildlife ("potential for subtle toxic effects in some species from some WST chemicals occurring within the NPDES discharge mixing zone from discharges of WST waste fluids to surface water") do BOEM and BSEE acknowledge *any* potential environmental impacts from offshore fracking and acidizing.

As illustrated in detail below, these analyses are inadequate under NEPA, and lack scientific and analytical integrity. Because the direct, indirect, and cumulative impacts of offshore well stimulation within the California OCS plainly may result in significant environmental impacts, BOEM and BSEE must now prepare a draft EIS to comply with NEPA's requirements and the *Environmental Defense Center v. Bureau of Safety and Environmental Enforcement* settlement agreement.

Unsupported and Inconsistent Assumption of Infrequent Use of WSTs

As a threshold matter, BOEM and BSEE improperly based their no impact determinations on the unsupported presumption that WSTs have been and will continue to be "infrequent activities." *See*, *e.g.*, Draft PEA at 4-31; *id*. at 4-60 ("Under Alternative 1, the use of any of the four WSTs included in the alternative is expected to have at most only limited or negligible impacts on potentially affected resources."). BOEM and BSEE

fail to provide any meaningful evidence for their assertion that offshore fracking and acidizing will only be infrequently used.

Notably, this conclusion has been undermined by the oil industry, which has consistently stated that it will not foreclose the use of WSTs in the future. In fact, the oil industry has clearly stated both specific plans, and general intentions, to continue well stimulation practices offshore California in the future. *See, e.g.*, Ken Dowd Declaration in Support of ExxonMobil Corporation's Motion for Leave to Intervene at 5, *EDC v. BSEE*, (No. 19-3) ("[W]ithin the next few months, ExxonMobil intends to apply in 2015 for an additional SPD in the SYU to drill a new well from the Harmony platform, which entails activities involving well stimulation technologies, including the pumping of acid to increase crude oil production from the new well."); *id.* at 6 ("In addition, ExxonMobil currently intends to invest substantially in its SYU leases for many years into the future. In the ordinary course of its operations, ExxonMobil will continue to evaluate and generate new opportunities to develop the SYU leases including but not limited to the drilling of new wells and stimulation of new and existing wells."); Notice of Motion and Motion for Leave to Intervene on Behalf of Defendant-Intervenor ExxonMobil Corporation, Memorandum of Points and Authorities at 1, *EDC v. BSEE*, (No. 19) ("ExxonMobil has future exploration and development plans for its substantial investments in offshore leases in the Pacific region that potentially involve well stimulation."); Declaration of Erik Milito in Support of the Motion to Intervene of American Petroleum Institute at 5, *EDC v. BSEE*, (No. 15-1) (referring to "future offshore plans of API members involving well stimulation methods").

The oil industry also claims that an injunction of WST would have a significant impact on its leases and interests, which undermines any assumption that the practice is likely to be infrequent. ExxonMobil Motion to Intervene at 6 ("The requested relief would have a significant detrimental impact on ExxonMobil's property, regulatory, and economic interests in its Santa Ynez Unit leases and permits. Specifically, the relief sought would enjoin the APDs and APMs upon which ExxonMobil has relied in continuing its oil and gas operations in its Santa Ynez Unit leases and enjoin work still left to be done under challenged permits. The relief sought would prevent ExxonMobil from implementing development activities under additional permits it has already obtained, and bring a halt to further exploration and development on ExxonMobil's investments in its offshore leases in Santa Barbara."); Milito Decl. at 5 ("API's members are directly affected by the Complaint's challenge both to permits already obtained by (or operated by) API's members on the California OCS, and to all pending and future offshore plans of API members involving well stimulation methods."). Moreover, industry openly relies on WST in order to facilitate development and production, which only indicates such practices are necessary to continue drilling on offshore platforms, and therefore likely to continue occurring. Motion to Intervene of American Petroleum

Institute at 9, *EDC v. BSEE* (No. 15) ("In addition, API members broadly rely on occasion on well stimulation technologies, including hydraulic fracturing and acidizing, to facilitate oil and gas exploration, development, and production throughout the federal OCS.").

In addition, and as discussed in more detail below, the assumption of infrequent WST use is directly at odds with other statements made throughout the draft PEA that the use of offshore fracking and acidizing, as well as other enhanced oil recovery techniques, is allowing the oil industry to produce oil and gas from previously inaccessible reserves, and is perpetuating the life of offshore oil platforms beyond their previously estimated life span.

Unlawful Reliance on Analysis for NPDES Revision

Another overarching and defining failure of BOEM and BSEE's analysis of direct environmental impacts is that several sections of the environmental impacts discussion improperly rely on the EPA California OCS National Pollution Discharge Elimination System ("NPDES") General Permit as a basis for its no impact conclusions. For example, in the discussion of marine mammal impacts, BOEM and BSEE state that no impacts would occur based on the EPA analysis associated with its recent revision of that permit. Draft PEA at 4-46. However, a non-NEPA document cannot satisfy a federal agency's obligations under NEPA. *Klamath-Siskiyou Wildlands Center v. Bureau of Land Management*, 387 F. 3d 989, 998 (9th Cir. 2004) (rejecting as "without merit" arguments that an agency may excuse itself from its NEPA hard look duty where a "facility operates pursuant to a state permit under the Clean Air Act."); *South Fork Band Council v. Dept. of Interior*, 588 F. 3d 718, 726 (9th Cir. 2009); *Makua v. Rumsfeld*, 163 F. Supp. 2d 1202, 1217 (D. Haw. 2001). Under NEPA, BOEM and BSEE must conduct their own independent environmental analysis.

Water Quality

The Draft PEA fails to adequately assess the impacts of WST discharges on water quality. As noted above, the analysis suffers a critical information gap regarding the composition of flowback fluids as opposed to injection fluids. It instead attempts to estimate impacts based on chemical concentrations in injection fluids and dilution of produced waste water, which provides no insight into impacts of flowback fluids. As Blue Tomorrow demonstrates, the composition of flowback fluid is distinct from injection fluid. Blue Tomorrow Expert Letter at 1-2 ("WST fluids prior to injection likely have substantially different chemistry and constituent concentrations than flowback fluids after a WST. During these treatments heavy metals, organics, and radioactive material can be mobilized from the formation, by chemicals in the injection fluid or by

the fracturing of the target formation, and mixed with the flowback fluids."). Specifically, flowback fluids are likely to contain additional pollutants and pose additional impacts, especially in the acidizing context. *Id.* at 2 ("[A]cid treatments (matrix acidizing, acid fracturing, and acid maintenance) use high concentrations of very strong acids such as HCL and HF acids to dissolve scaling and clogging of the well bore, and to dissolve the formation rock itself to increase connectivity and permeability within the formation to increase production. After the acid treatment fluids return to the surface, they can contain very high levels of dissolved solids and heavy metals and have been reported to have pH in the range of 0 to 3.). These pollutants are "not present in injection fluids," *id*, rendering the agencies' analysis of impacts of WST discharges based on injection fluids inadequate.

Moreover, the agencies' provide no direct evidence to conclude that WST discharges have no impacts on ecological resources. Their reliance on the CCST study is insufficient, which itself acknowledges a lack of data. *Id.* at 2 ("The 2015 CCST assessment includes literature review of studies of ecological conditions and contamination in the marine environment around California offshore platforms, and laboratory investigation of the toxicity of produced water discharges on the marine environment. However, the EA does not include direct evidence to support the determination that no ecological resources will be effected by the discharge of WST flowback fluids.").

The Draft PEA acknowledges there is a "lack of toxicity data for many constituents of WST fluids." Draft PEA at 4-30. However, it fails to sufficiently address this data gap, or provide adequate information to meaningfully address impacts of WST discharges on the marine environment. Blue Tomorrow Expert Letter at 3 ("[T]he information cited in the EA regarding the eco-toxicity of chemicals found in WST stimulation fluids is insufficient to justify that there is no potential to impact ecological resources."). The agencies should instead determine "[a]cute and chronic toxicity data for well stimulation chemicals, as well as chemicals identified in flowback fluids that may be discharged to the ocean" in order to evaluate impacts. *Id.* (*quoting* CCST 2015 at 103).

In addition, the Draft PEA fails to sufficiently address whole effluent toxicity ("WET"). As Blue Tomorrow explains, while some analysis was conducted as to "individual toxic effects" of WST fluids, "[t]here are both cumulative and interaction (or synergistic) affects that should be considered in assessing the toxic effects of a fluid with multiple toxic constituents." *Id.* The Draft PEA simply fails to conduct this analysis. The WET testing performed under the NDPES General Permit is inadequate because it is not timed with WST discharges and is performed infrequently. *Id.* ("However, WET testing is performed quarterly, and will likely not capture the toxicity effects of from

WST fluid discharges, as "the timing of WET tests is not linked to well stimulation events in the NPDES permit" (CCST 2015, page 71). Furthermore, if results from WET tests indicate no observable effects, the testing frequency is reduced from quarterly to annual WET tests (NPDES CAG280000 2013). Due to the infrequency of WET testing and its lack of linkage with WST discharges, the EA's assumption that previous results from WET testing in the OCS has not demonstrated impacts from WST operations is flawed."). Therefore, the Draft PEA is inadequate for its failure to address potentially significant impacts to water quality.

The Santa Barbara Channel, Protected Lands and Waters, and Endangered Species

As noted above, the "context" of a proposed action is one of two key factors in determining "significance" of environmental impacts and the requirement to prepare an EIS. Context "means the significance of the action must be analyzed in several contexts such as society as a whole (human, national), the affected region, the affected interests, and the locality." 40 C.F.R. § 1508.27(a). The "context" of a proposed action will often also have significant overlap with the "intensity" factors enumerated under CEQ's regulations, including the presence of threatened and endangered species, ecologically important areas, and other considerations.

In this case, the environmental setting, or context, of offshore oil drilling in Southern California federal waters generally, and the use of WST in particular, further undermines BOEM and BSEE's blanket determination of no or *de minimus* environmental impacts in the draft PEA. In particular, the large majority of California's offshore oil platforms in federal waters are located in the Santa Barbara Channel. As detailed in our 2013 "Dirty Water" report,[4] the Santa Barbara Channel harbors extraordinary biological diversity, so much so that it is dubbed "the Galapagos of North America." Blue, fin, and humpback whales, and the southern sea otter are among the threatened and endangered species that depend on the Channel for their survival and recovery.

Reflecting this environmental importance, many of the waters and islands of the Santa Barbara Channel are specially designated and protected, including the Channel Islands National Park and Channel Islands National Marine Sanctuary. Specific platforms from which WST has been conducted are in direct proximity to these protected zones. The Santa Clara Unit (platforms Gail and Gina in particular), for example, lies in close proximity to the Marine Sanctuary boundaries, while platforms A, B, and C are

[4] Brian Segee & Elise O'Dea, *Dirty Water: Fracking Offshore California*, (2013), Available at http://www.environmentaldefensecenter.org/wp-content/uploads/2015/03/DirtyWater.pdf

directly adjacent to the Santa Barbara Channel Federal Ecological Preserve. In addition, the Channel is home to a network of state and federal Marine Protected Areas ("MPAs"). There are thirteen MPAs at the Channel Islands and five along the mainland coast from Point Conception to the Goleta Slough.

The Channel, as well as the waters off Long Beach and the northern Orange County coastal cities of Huntington Beach and Seal Beach, also serves as a primary economic engine for southern California tourism, fisheries, and other industries. For example, the commercial fishing industry in Santa Barbara alone generated over $11 million on average in annual earnings between 1980 and 2013. Lisa Wise Consulting, Inc., *Commercial Fisherman of Santa Barbara, 2014 Commercial Fisheries Economic Impact Report* at 2 (April 2015). In 2013, Santa Barbara had the highest earnings in the state of California with respect to seven species, including: the red sea urchin, California spiny lobster, red rock crab, yellow rock crab, giant red sea cucumber, white seabass, grass rockfish. *Id.* at 3–4. Working waterfronts also attract tourism. *Id.* at 4.

The threat posed by offshore drilling to the local economy was recently vividly illustrated by the failure of Plains All-American Pipeline LLP, Line 901 at Refugio State Beach. Key fishing areas were closed from Cañada de Alegria to Coal Oil Point up to 6 miles offshore, and two popular state beaches, Refugio and El Capitan, were closed during a busy holiday weekend, and remained closed for over a month. Six class action lawsuits were filed in response to the spill on behalf of members of the fishing and tourism industries, platform workers, and property owners. *See* Consolidated Complaint, *Stace Cheverez v. Plains All American Pipeline, LP*, 2:15-cv-04113 (C.D. Cal. Dec. 15, 2015). These lawsuits demonstrate that the risks associated with offshore oil result in considerable economic harm. *Id.* at 11 ("In Santa Barbara, these environmental impacts translate to profound economic impacts. In the short term, the oil from Defendants' ruptured pipeline closed fishing grounds and shellfish areas, and caused many cancelled reservations from tourists who otherwise would have spent their money on hotels, restaurants, kayaking or surf trips, fishing charters, and in the region's retail stores.").

While the extent of economic damage is still being calculated, a handful of examples illustrate just the beginning of damages that were suffered. *Id.* at 26 (a community seafood company reported 350 cancelled seafood shares, resulting in over $6,5000 revenue loss in just the one week following the spill); *id*. at 42 (fishing company reported that its squid fishing can generate up to $30,000 in a single night, and the spill resulted in closures of squid fishing areas on which the company relies); *id.* at 11 (kayaking company reported 25 cancellations following the spill, resulting in a loss of approximately $3,000).

Under NEPA, the concentrated presence of threatened and endangered species, proximity to protected areas such as the Channel Islands National Park and National Marine Sanctuary, and overall centrality of the Santa Barbara Channel to the daily fabric of its coastal communities, our economies, and overall way of life are a central factor to consider in deciding whether to prepare an EIS. In areas such as the Santa Barbara Channel, even allegedly "minimal" environmental risks can be considered significant enough to compel the need for an EIS.

In the draft PEA, BOEM and BSEE mention the presence of these areas, but fail to adequately acknowledge the unique environmental, economic, and social importance of the Santa Barbara Channel, the risks posed to the Channel by offshore fracking and acidizing, and the avenues to avoid or minimize those risks. This deficiency is further underscored by the fact that the Ninth Circuit Court of Appeals has already specifically recognized the importance of this environmental context in relation to OCS oil and gas production offshore California. *California v. Norton*, 311 F. 3d 1162, 1176–77 (9th Cir. 2002) (finding substantial evidence of extraordinary circumstances including the potential adverse impacts on threatened and endangered species including the southern sea otter; the potential adverse effects on ecologically significant or critical areas including the Channel Islands National Marine Sanctuary; and the existence of highly controversial environmental effects of offshore oil drilling).

Public Controversy

Oil drilling offshore California generally, and the use of offshore fracking and acidizing in particular, also has "highly controversial environmental effects." *See id.* §46.215(c); *Norton*, 311 F.3d at 1177 ("That there has been continuous and significant public controversy over the environmental effects of offshore oil activities in California for the past thirty years, and that there is significant public controversy over these lease extensions in particular is beyond debate."). Offshore drilling in the Santa Barbara Channel has been highly controversial since it was first proposed many decades ago, and the controversy has not abated in light of numerous spills and other accidents in the region, including the 1969 Santa Barbara oil spill and 2015 Refugio State Beach oil pipeline spill. Well stimulation in the Santa Barbara Channel has only further added to the continuing public controversy, and continued local, regional, and national public debate.

Indirect Impacts

CEQ NEPA regulations require agencies to consider the indirect effects of a proposed action, also known as secondary impacts. Indirect effects are defined as those "which are caused by the action and are later in time or farther removed in distance, but

are still reasonably foreseeable." 40 C.F.R. § 1508.8(b); *Border Power Plant Working Group v. Department of Energy*, 260 F. Supp. 2d 997 (S.D. Cal. 2003). As acknowledged in the draft PEA, "[a]dvances in WSTs and the availability of enhanced oil recovery (EOR) techniques have allowed for continued production from onshore and offshore reservoirs where primary recovery has begun to decline as a result of declining reservoir pressures . . . The use of WSTs may support the continued recovery of oil as primary recovery declines with the 43 active lease areas." Draft PEA at 1-3–1-4. Despite this acknowledgment, BOEM and BSEE neglect to consider the associated environmental impacts and risks associated with extending the life of aging offshore oil platforms beyond their intended life span.

For example, DOI estimated that Platforms Gina and Gina, located off the City of Oxnard's coastline, would together produce 52 million barrels of crude oil and 42 billion cubic feet of natural gas over a period of approximately 20 years. *See* Platform Gilda and Platform Gina Project Environmental Impact Report/Environmental Assessment, Volume I (May 1980) (Prepared by City of Oxnard and U.S.G.S.), at p. 3.1-2; *id*. at Figure 3.5-1 (anticipated production schedule for Platform Gina); *id*. at Figure 3.5-2 (Anticipated Production Schedule for Platform Gina—Repetto Formation). Platform Gina's estimated lifespan was even more brief, at only 18 years. *Id*. at p. 4.3-9. The analysis contained no consideration of the use of offshore fracking and acidizing, but did estimate that development of the Monterey Foundation could extend the life of Platform Gilda by an additional 5 years.

Platform Gina was installed in 1980, 36 years ago, while Platform Gail was installed in 1987, 29 years ago. Draft PEA, at Table 3-1. Thus, both are already operating well beyond the estimated life span and the 20 year environmental analysis associated with that assumption. WST would only increase this life span further of these and other Southern California OCS production platforms, and yet BOEM and BSEE have never addressed the increased environmental impacts and risks associated with this extension, or determined whether the obvious design and engineering issues associated with reliance on this aging infrastructure.

Cumulative Impacts

One of the ten factors agencies must consider when assessing significance is "whether the action is related to other actions with individually insignificant but cumulatively significant impacts." 40 C.F.R. § 1508.7. A cumulative impact on the environment "results from the incremental impact of the action when added to other past, present, and reasonably foreseeable actions regardless of what agency . . . or person undertakes such actions." *Id*. Courts have thus consistently held that NEPA's cumulative effects requirements apply to EAs as well as EISs. *See Kern v. United States*

Bureau of Land Management, 284 F.3d 1062, 1076 (9th Cir. 2002) ("[A]n EA may be deficient if it fails to include a cumulative impact analysis or to tier to an EIS that has conducted such an analysis."); *Grand Canyon Trust v. F.A.A.*, 290 F.3d 339, 347 (D.C. Cir. 2002), as amended (Aug. 27, 2002). As stated in that case, "the consistent position in the case law is that, depending on the environmental concern at issue, the agency's EA must give a realistic evaluation of the total impacts and cannot isolate a proposed project, viewing it in a vacuum." *Id*. at 342.

Here, however, BOEM and BSEE have failed to adhere to this basic NEPA principle, and thus undermined one of the fundamental purposes of NEPA review, "to insure that the agency considers all possible courses of action and assesses the environmental consequences of each proposed action." *Sierra Club v. Peterson*, 717 F.2d 1409, 1414 (D.C. Cir. 1983). A searching inquiry into potential cumulative effects in this instance is particularly imperative in light of: (1) the extensive existing oil and gas infrastructure and operations already present in the Santa Barbara Channel and off the Long Beach and northern Orange County coastline; (2) the fact that the well stimulation is acknowledged to facilitate yet more oil and gas operations that would otherwise not occur; and (3) the extraordinary natural habitats and wildlife species within the Santa Barbara Channel.

The draft PEA does not provide any insight into the past, present, and reasonably foreseeable actions that would help portray a "realistic evaluation of the total impacts" of the proposed action. *Grand Canyon Trust*, 290 F.3d at 342. The draft PEA includes "cumulative impacts" sections with respect to each alternative in which it purports to address "past, current, and reasonably foreseeable future activities" of the action. However, the analysis is "conclusory" and "vague" and thus inadequate under NEPA. *See Te-Moak Tribe of W. Shoshone of Nevada v. U.S. Dep't of Interior*, 608 F.3d 592, 603 (9th Cir. 2010); *Lands Council v. Powell*, 379 F.3d 738, 745 (9th Cir. 2004)(NEPA analysis "must give a sufficiently detailed catalogue of past, present, and future projects, and provide adequate analysis about how these projects, and differences between the projects, are thought to have impacted the environment."); *Grand Canyon Trust*, 290 F.3d at 342 (*quoting Coalition for Sensible Transportation v. Dole*, 826 F.2d 60, 70–71 (D.C. Cir. 1987)) ("it makes sense to consider the 'incremental impact' of a project for possible cumulative effects by incorporating the effects of other projects into the background 'data base' of the project at issue.").

For example, with respect to Alternative 1, the draft PEA lists "past, current, and reasonably foreseeable future activities" that contribute to cumulative impacts, as including "oil and gas development and production activities in Federal and State waters as well as onshore; runoff from onshore industries, agriculture, transportation (fossil fuel combustion products), urban development, and sewage treatment plant discharges;

commercial and recreational fishing; commercial and recreational vessel traffic; and recreation and tourism." Draft PEA at 4-60. It notes, in one general sentence, that these activities may impact certain resources including air and water quality. Based on this conclusory statement, the draft PEA then jumps to the conclusion that because the expected use of WSTs is likely to have "negligible impacts" that are "temporary, localized . . . and infrequent," Alternative 1's impacts "are not expected to result in any measurable increases in cumulative effects on resources or socioeconomic/sociocultural conditions of the project area." *Id.* The remaining cumulative impacts analysis largely relies on the analysis for Alternative 1, and is even more cursory. Draft PEA at 4-62, 4-63, 4-66–67.

This summary conclusion does not include the detailed information that NEPA requires, leaving the public in the dark as to the true impacts of the action. *Neighbors of Cuddy Mountain v. United States Forest Service*, 137 F.3d 1372, 1379 (9th Cir. 1998) ("To 'consider' cumulative effects, some quantified or detailed information is required. Without such information, neither the courts nor the public, in reviewing the [agency's] decisions, can be assured that the [agency] provided the hard look that it is required to provide."). Even a conclusion that there are no cumulative impacts must be supported by more detail than the draft PEA provides. *See Klamath-Siskiyou Wildlands Ctr.*, 387 at 996. In addition, this analysis improperly hinges on the unfounded assumption of negligible direct impacts, without actually analyzing cumulative impacts. *Te-Moak Tribe*, 608 F.3d at 604 ("The EA's discussion of the [action's] direct effects in lieu of a discussion of cumulative impacts is inadequate.").

As a specific example of inadequate cumulative effects analysis, the EA fails to analyze impacts associated with oil infrastructure, including pipelines, processing plants and the risk of oil spills. The Refugio Oil Spill is an example of the extent of such potential impacts. On May 19, 2015, the Plains All-American Pipeline 901 suffered a massive leak, due to external corrosion, resulting in over 140,000 gallons of crude spilling from onshore Gaviota Coast, onto the beach and into the ocean. Pipeline and Hazardous Materials Safety Administration, *Preliminary Findings Report: Plains Pipeline, LP, Failure on Line 901* (Feb. 17, 2016). The spill resulted in hundreds of dead birds and mammals and more injured, 150 miles of coastline contaminated, two State parks closed, and 138 square miles of fishing grounds closed. Pipeline 901 delivers crude that originates from seven offshore oil platforms in the Channel, including from Platforms Heritage, Harmony and Hondo (operated by ExxonMobil); Hidalgo, Harvest and Hermosa (operated by Freeport McMoran); and Holly (operated by Venoco). Offshore WST is known to occur on at least three of these platforms. Offshore WST enhances production and extends the life of offshore oil platforms, necessitating continued operation of oil infrastructure, and posing additional threats. The draft PEA

fails to analyze the impacts of existing and future oil infrastructure or assess the incremental impacts of WSTs.

In addition, the draft PEA does not contain any meaningful information regarding the extent of "routine" acidizing, and the estimated impacts on the environment this practice may have. BOEM and BSEE apparently consider essentially all treatments using acid to be "routine," as the draft PEA states that only two matrix acidizing treatments were conducted on the California OCS between 1985 and 2011, and that "the rest would be currently classified as routine well maintenance treatments." Draft PEA, at p. ES-8; p. 4-3. This information appears to conflict with information EDC has compiled through its own review of BSEE records obtained through FOIA, including specific permits that were challenged in our lawsuit. *See, e.g.* Jan. 3, 2011 APM at Platform Harmony (authorizing "acid stimulate" with 17,000 gallons 15% HCL and 26,000 gallons 12-3 mud acid (12% HCL + 3% HF); March 22, 2013 APM at Platform Harmony (authorizing "acid stimulate" with 75,000 gallons 15% HCL); December 2, 2013 APM at Platform Gilda (authorizing "acid stimulation"). Whatever label is ascribed to the practices authorized under these permits, their use should have been considered in the cumulative impacts analysis and was not.

Compounding BOEM and BSEE's inadequate cumulative impacts analysis is the bizarre contention that alternative 4, which would prohibit WST, will have greater impacts than the use of offshore fracking and acidizing, based on the baseless contention that it "may necessitate the drilling and production of new wells offshore and/or onshore, increase WST use at onshore wells, and/or increase the need to import more gas and oil." Draft PEA, at p. 4-67. Notably, BOEM and BSEE fail to provide any support for this conclusion. Moreover, this statement once again highlights the unsupported presumption permeated throughout the draft document that offshore fracking and acidizing will have no impacts. Only by mischaracterizing, discounting, and willfully ignoring these local impacts, can BOEM and BSEE even consider claiming that prohibiting their use will actually result in greater environmental impacts.

Preparation of an EIS is Required

An EIS "must be prepared if substantial questions are raised as to whether a project may cause significant degradation of some human environmental factor." *Klamath Siskiyou Wildlands Center v. Boody*, 468 F.3d 549, 562 (9th Cir. 2006). "[A] plaintiff need not show that significant effects will in fact occur, but if the plaintiff raises substantial questions whether a project may have a significant effect, an EIS must be prepared." *Idaho Sporting Congress v. Thomas*, 137 F.3d 1146, 1150 (9th Cir. 1998). "This is a low standard." *Klamath Siskiyou*, 468 F.3d at 562.

The [agency] cannot avoid preparing an EIS by making conclusory assertions that an activity will have only an insignificant impact on the environment. *Alaska Ctr. for Env't v. United States Forest Serv.*, 189 F.3d 851, 859 (9th Cir. 1999). If BOEM and BSEE opt not to prepare an EIS, the agencies must put forth a "convincing statement of reasons" that explain why the project will impact the environment no more than insignificantly. *Blue Mountains Biodiversity Project v. Blackwood*, 161 F.3d 1208, 1212 (9th Cir. 1998). This account proves crucial to evaluating whether the agencies took the requisite "hard look" at the potential impact of offshore fracking and acidizing.

Thus, in this case, the agency's failure to fully review all direct, indirect, and cumulative impacts renders the draft PEA deficient. As such, BOEM and BSEE cannot issue a FONSI. Without the required review under NEPA, any decision not to prepare an EIS is without sufficient evidentiary support.

Endangered Species Act ("ESA")

The ESA is "the most comprehensive legislation for the preservation of endangered species ever enacted by any nation." *TVA v. Hill*, 437 U.S. 180 (1978). Its fundamental purposes are "to provide a means whereby the ecosystems upon which endangered species and threatened species depend may be conserved [and] to provide a program for the conservation of such endangered species and threatened species . . . ." 16 U.S.C. § 1531(b). To achieve these objectives, the ESA directs the U.S. Fish and Wildlife Service ("FWS") or National Marine Fisheries Service ("NMFS") to determine which species of plants and animals are "threatened" and "endangered" and place them on the endangered species list. *Id*. § 1533. An "endangered" or "threatened" species is one "in danger of extinction throughout all or a significant portion of its range," or "likely to become endangered in the near future throughout all or a significant portion of its range," respectively. *Id*. § 1532(6) & (20).

Once a species is listed, the ESA provides a variety of procedural and substantive protections to ensure not only the species' continued survival, but its ultimate recovery. One central protection, section 7(a)(2), mandates that all federal agencies avoid actions that: (1) jeopardize listed species; or (2) destroy or adversely modify designated critical habitat. *Id*. § 1536(a)(2). To comply with these section 7(a)(2) safeguards, the federal agency taking action and FWS take part in a cooperative analysis of potential impacts to listed species and their designated critical habitat known as a consultation process. Federal agencies must consult with FWS or NMFS when their actions "may affect" a listed species or designated critical habitat. 50 C.F.R. § 402.14(a). Federal agency actions include those projects "authorized, funded, or carried out by such agency." *Id*. "Action area" is defined broadly under the ESA implementing regulations to include "all areas to be affected directly or indirectly by the Federal action and not merely the

immediate area involved in the action." 50 C.F.R. § 402.02.

To facilitate the consultation process, the federal agency proposing a project must prepare a "biological assessment," or BA, which identifies listed species in the area and evaluates the potential effects of the proposed action. *Id*. §§ 402.02, 402.12. At the completion of consultation, FWS or NMFS prepares a "biological opinion" as to whether the action jeopardizes the species or destroys or adversely modifies critical habitat and, if so, suggests "reasonable and prudent alternatives." 16 U.S.C. § 1536(b)(3)(A). Both agencies must "use the best scientific and commercial data available" during the consultation process. *Id*. § 1536(a)(2); 50 CFR § 402.14(d).

As described in detail above, BOEM and BSEE have concluded in the draft PEA that the use of offshore fracking and acidizing will have no impacts, including impacts on the many threatened and endangered species found in the Santa Barbara Channel, as well as at the Long Beach platforms. The draft PEA states that the development of the EA "will facilitate DOI meeting other environmental requirements *related to future authorizations*, such as Endangered Species Act, Marine Mammal Protection Act, and Coastal Zone Management Act requirements." Draft PEA, at p. 1-5. Accordingly, it appears that BOEM and BSEE do not intend to initiate ESA consultation on this programmatic environmental assessment.

This failure to initiate and complete consultation with FWS (in relation to the southern sea otter and other species) and NMFS (in relation to blue whale, fin whale, humpback whale, sea turtles, and other species) in relation to this PEA would be a clear violation of the ESA, as any "no effect" determinations are not supported by the available evidence and best scientific information available.

Coastal Zone Management Act ("CZMA")

The CZMA was enacted in 1972 in order to provide comprehensive, coordinated planning for the protection and beneficial uses of the "coastal zone," defined to include land near the shorelines of coastal states, as well as coastal waters extending seaward to the limits of the United States territorial sea. 16 U.S.C. § 1451, 1452, 1453(1). The territorial sea for coastal states bordering the Atlantic and Pacific Oceans extends three geographical miles seaward from the coastline, while submerged federal lands that lie beyond this 3-mile limit constitute the "outer continental shelf." 42 U.S.C. §§ 1302, 1311. The CZMA closely interacts with the OCSLA, which among things establishes detailed processes and requirements for federal oil and gas leasing and permitting activities in the OCS. 43 U.S.C. §§ 1331–1356.

In passing the CZMA, Congress found that the "increasing and competing

demands upon the lands and waters of our coastal zone" had "resulted in the loss of living marine resources, wildlife, nutrient-rich areas, permanent and adverse changes to ecological systems, decreasing open space for public use, and shoreline erosion." 16 U.S.C. § 1451(c). Accordingly, it placed particular emphasis on the objective of preserving coastal natural resources "for this and succeeding generations." 16 U.S.C. § 1452(1).

One of the CZMA's fundamental mechanisms to achieve this overarching objective was to provide coastal states with oversight over activities in federal waters where those states have adopted a Coastal Management Program ("CMP") to manage coastal land and water uses. The CMP's purpose is to encourage coastal states to manage their coastal resources in accordance with specific national priorities, including protection of natural resources and water quality. 16 U.S.C. § 1452. In coastal states with federally approved CMPs, federal government actions (including permitting or licensing) proposed in federal waters are subject to state oversight prior to approval.

This oversight process, known as "consistency review," is a "unique federal-state coordinated regulatory process . . . which grants coastal states which elect to participate in the CZMA program the ability to regulate federal activities that affect their coastal zone." *CZMA Federal Consistency Regulations Final Rule*, 71 Fed. Reg. 75,864 (Dec. 19, 2016). The "federal consistency program is a cornerstone of the CZMA program and a primary incentive for State's participation." *Id*.; *California v. Norton*, 150 F. Supp. 2d 1046 (N.D. Cal. 2001), *aff'd* 311 F.3d 1162 (9th Cir. 2002). The National Oceanic and Atmospheric Administration ("NOAA") certified the California CMP in 1978.

Regulations implementing the CZMA consistency requirement apply to "all Federal agency activities . . . affecting any coastal use or resource." 15 C.F.R. § 930.30. "Federal agency activity," in turn, is defined broadly to include "a range of activities where a Federal agency makes a proposal for action initiating an activity or series of activities when coastal effects are reasonably foreseeable." *Id*. § 930.31(a). Federal permits authorizing WSTs affect coastal uses and resources and are therefore subject to CZMA consistency review. *See California v. Norton*, 150 F. Supp. 2d at 1052–54 (concluding that the granting of a request to suspend an offshore oil lease is subject to CZMA consistency review because it is a federal activity affecting the coastal zone).

In this instance, BOEM and BSEE have identified the proposed action as the general approval of offshore fracking and acidizing. As California Coastal Commission staff has repeatedly communicated to BOEM and BSEE, these activities have not

previously undergone CZMA consistency analysis. Accordingly, that analysis must be conducted now, rather than delaying again to future site-specific permit applications.

Conclusion

EDC and Surfrider Foundation again thank you for this opportunity to comment on the draft PEA. In light of the numerous NEPA shortcomings discussed in this letter and overarching failure of the draft PEA to adequately analyze the environmental impacts and risks associated with offshore fracking and acidizing, we again request that BOEM and BSEE instead initiate preparation of an EIS that acknowledges the significant environmental impacts and risks associated with offshore fracking and acidizing, and that provides a more detailed and thorough analysis of those impacts and risks.

Sincerely Yours,

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ATTACHMENTS

LIST OF ATTACHMENTS

March 22, 2016

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This review of the Programmatic Environmental Assessment of the Use of Well Stimulation Treatment on the Southern California Outer Continental Shelf (EA) has been conducted on behalf of the Environmental Defense Center. Blue Tomorrow, LLC is an environmental consulting company that specializes in assessing environmental impacts from oil and gas operations. The EA has been reviewed along with the literature cited by the Lead Agencies (Bureau of Ocean Energy Management and Bureau of Safety and Environmental Enforcement), and this review discusses the following concerns with the EA evaluation:

- Evaluation of discharge toxicity focuses on chemistry of well stimulation treatments (WST) injection fluids and not the composition of flowback fluids
- Ecological impacts from discharges of WST flowback fluids have not been studied
- Many constituents in WST have unknown toxicity
- Insufficient justification for the determination that whole effluent toxicity (WET) testing required through the NPDES permit will limit the risk from WST discharges

As a result of the flawed assumptions highlighted in this discussion, the EA evaluation is insufficient to support the determination that no significant impacts are likely to occur from the use of WST on 23 platforms currently in operation on the Southern California Outer Continental Shelf (OCS) Planning Area. The potential uncertainties and impacts discussed below should be evaluated to adequately assess the potential for environmental impacts to occur as a result of the proposed action.

Evaluation of discharge toxicity focuses on chemistry of WST injection fluids and not the composition of flowback fluids

A fundamental problem with assessing the toxicity of WST is the lack of information on the composition of discharged fluids. During WSTs additional constituents are being mobilized from the formation and their chemistry and toxicity are unknown. Quantifying the risk from discharging these fluids is not possible without this information.

WST fluids prior to injection likely have substantially different chemistry and constituent concentrations than flowback fluids after a WST. During these treatments heavy metals, organics, and radioactive material can be mobilized from the formation by chemicals in the injection fluid or by the fracturing of the target formation and mixed with the flowback fluids (Abdullah, et.al. 2016; Nelson, et.al 2014; Fischer et.al. 2014). For example, acid treatments (matrix acidizing, acid fracturing, and acid

maintenance) use high concentrations of very strong acids such as HCl and HF acids to dissolve scaling and clogging of the well bore, and to dissolve the formation rock itself to increase connectivity and permeability within the formation to increase production. After the acid treatment fluids return to the surface, they can contain very high levels of dissolved solids and heavy metals and have been reported to have pH in the range of 0 to 3 (Abdullah, et.al. 2016). These pH levels are similar to those found in acid mine drainage that is known to dissolve high levels of heavy metals from the source rock.

The EA evaluates the potential environmental impact of WST by assessing what little information is known about hydraulic fracturing fluid, and states: "With respect to WST fluid constituents in discharges, concentrations for all constituents can be estimated from quantities injected and levels of dilution in produced water, both of which are known quantities." Additionally, the EA states that the acids in flowback fluids would be "largely consumed and neutralized" and therefore "would produce no effects on water quality or marine life from discharges mixed with produced water". The approach of estimating constituent concentrations in discharged flowback fluid used in the EA is insufficient for the reasons discussed above. The interaction of injection fluid chemicals with the formation will result in substantially different constituents and concentrations in flowback fluids compared to injection fluids, especially in the case of acid treatments where flowback fluids can contain very high levels of dissolved solids and toxic heavy metals not present in injection fluids.

Ecological impacts from discharges of WST flowback fluids have not been studied

The EA determined that no WST-related impacts to ecological resources are expected beyond the potential for subtle sublethal impacts in some species from some WST chemicals occurring within the 100m mixing zone. To support this determination, the EA relies largely on information presented in Volume III of the Independent Scientific Assessment of Well Stimulation in California (CCST 2015). The 2015 CCST assessment includes literature review of studies of ecological conditions and contamination in the marine environment around California offshore platforms, and laboratory investigation of the toxicity of produced water discharges on the marine environment. However, the EA does not include direct evidence to support the determination that no ecological resources will be affected by the discharge of WST flowback fluids. The authors of the 2015 CCST case study clearly state: **"**Direct evidence for impacts of well stimulation fluid discharge into the marine environment is not available**.** The available information only provides a rough idea concerning the magnitude of stimulation activity conducted offshore, and the composition of stimulation flowback fluids is not known. There are no studies of stimulation or flowback fluids effects on the marine environment (Houseworth & Stringfellow, 2015)**."** As a result of the absence of scientific studies of impacts to the marine environment from WST waste discharges, the EA evaluation is insufficient to support the conclusion that no WST-related impacts to ecological resources are expected to occur.

The EA states that the ecological toxic effects of discharging WST flowback fluids will be mitigated by diluting the flowback fluids with produced water before discharge. Produced water has been shown to be lethal to larva of the Watersipora subtorquata (a sessile filter feeding animal when mature) at concentrations of 10% produced water mixed in seawater and sub-lethal effects at concentrations around 1% (Raimondi & Boxshell 2002). As discussed in the previous section, WST flowback fluids may contain different constituents and different concentrations than injection fluids or produced water from

regular production. Mixing (diluting) these fluids with the already potentially harmful produced water would likely result in different ecological toxicity effects. Depending on the dilution of WST fluids with produced water, the impacts from produced water discharges may be increased compared to those that have been studied and documented. The ecological impacts from WST flowback fluids and whether or not they extend beyond the 100m mixing zone is unknown and should be investigated.

Many constituents in WST have unknown toxicity

The EA confirms that potential effects on marine life are not fully understood, "due, in part, to the lack of toxicity data for many constituents of WST fluids" (EA, 2016). Included in the EA is a toxicity screen that was conducted through the 2015 CCST case study, which compared diluted values of chemicals found in hydraulic fracturing and acidizing stimulation fluids with acute and chronic toxicity values for 90 marine species. As flowback compositions were not available, the authors used stimulation fluid compositions contained in DOGGR public disclosure reports, which limits conclusions that can be drawn from this evaluation (as previously discussed). Nonetheless, this screening utilized all available data through the U.S. EPA ECOTOX of which only a fraction of chemicals had toxicity data for marine organisms (26 of the 33 chemicals screened in the hydraulic fracturing fluid; and 5 of the 17 chemicals screened in the acidizing case study) (Houseworth & Stringfellow, 2015).

Furthermore, Houseworth and Stringfellow recommend that, "an assessment of the discharge of wastewater well stimulation fluids into the ocean should be done. Acute and chronic toxicity data for well stimulation chemicals, as well as chemicals identified in flowback fluids that may be discharged to the ocean, should be determined to provide a basis for understanding environmental effects of this discharge, just as these types of studies have been performed to assess the impacts of produced water discharge (Houseworth & Stringfellow, 2015)." As such, the information cited in the EA regarding the eco-toxicity of chemicals found in WST stimulation fluids is insufficient to justify that there is no potential to impact ecological resources.

Insufficient justification for the determination that WET testing required through the NPDES permit will limit the risk from WST discharges

Some of the constituents used in hydraulic fracturing fluids that have toxicity data were reviewed in the EA with respect to their individual toxic effects (see above). While this is an adequate initial screening of individual chemicals, it does not evaluate the toxicity of the whole fluid (the whole effluent toxicity). There are both cumulative and interaction (or synergistic) effects that should be considered in assessing the toxic effects of a fluid with multiple toxic constituents (Cedergreen, 2014).

One way of assessing the WET of discharges is to perform a WET test using EPA methodology for estimating the acute and chronic toxicity of effluents and receiving waters to marine organisms. Under the National Pollutant Discharge Elimination System (NPDES) permit that is currently in place for the 23 offshore platforms, WET testing involves the short-term exposure of three test organisms (red abalone, giant kelp, and topsmelt) to a 24-hour composite effluent sample (multiple samples collected over 24 hours and combined into one sample) recovered from well completion, treatment, and workover operations (NPDES General Permit CAG280000, 2013). However, WET testing through NPDES is

performed quarterly, and will likely not capture the toxicity effects of WST fluid discharges, as "the timing of WET tests is not linked to well stimulation events in the NPDES permit (Houseworth & Stringfellow, 2015)." Furthermore, if results from WET tests indicate no observable effects, the testing frequency is reduced from quarterly to annual WET tests (NPDES General Permit CAG280000, 2013). Due to the infrequency of WET testing and its lack of linkage with WST discharges, the EA's assumption that previous results from WET testing in the OCS has not demonstrated impacts from WST operations is flawed.

Conclusion

The information presented in the EA is insufficient to support the determination that no significant impacts are likely to occur from the use of WST on 23 platforms currently in operation on OCS Planning Area. There are several concerns that have not been addressed through the EA including the ones discussed in this review. The 2015 CCST Case Study on California Offshore Petroleum Production, Well Stimulation, and Associated Environmental Impacts (cited throughout the EA to support its determination) states:

"Significant data gaps include data concerning the occurrence of well stimulation treatments, information on stimulation-fluid composition, treatment intervals and depths, flowback quantities and compositions, and ultimate disposition of flowback. Data relevant to these issues are insufficient and inadequate for quantitative impact assessments. In some cases, such as flowback quantities and compositions, the information is completely absent. In addition, no studies have been conducted on the toxicity and impacts of well stimulation fluids discharged in federal waters to the marine environment (Houseworth & Stringfellow, 2015)." This statement highlights the current inability to conduct an assessment of environmental impacts that may result from WST activities in the OCS Planning Area based on a lack of information. Therefore, the supporting evidence is inadequate to justify the conclusion that no environmental impacts are expected to occur as a result of the proposed action.

Further testing is needed to evaluate the potential impacts to marine organisms. WST flowback fluids (acidizing and hydraulic fracturing) should be analyzed and the composition of these fluids presented in an Environmental Impact Statement. Additionally, WET testing should be done on WST flowback fluids to evaluate the toxicity of these discharges. These WET tests should dilute the WST flowback fluids with sea water only and not additional dilution with other sources of produced water. Unless the composition and toxicity of WST flowback fluids is known, impacts that may result from WST activities to water quality and ecological resources cannot be sufficiently evaluated.

Respectfully,

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Toxicity of acidization fluids used in California oil exploration

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ABSTRACT

There has been considerable public interest regarding the toxicity of chemicals used in hydraulic fracturing, but little is known about its sister technique, acidizing. Little to no research has been done on what the chemicals of acidization are and what impact they could have on humans and the environment. This paper discusses the differences between three acidizing techniques (acid maintenance, matrix acidization, and acid fracturing) and quantifies the amounts of the chemicals used for each. Washington State's Quick Chemical Assessment Tool is used to identify F-graded toxins, which are known carcinogens, mutagens, reproductive toxins, developmental toxins, endocrine disruptors, or high acute toxicity chemicals. The analysis of the present data shows that there have been over 600 instances of acidizing in urbanized Southern and Central California from April 2013 to August 2015. Although most of the chemicals of acidizing are similar to hydraulic fracturing, those used most frequently are different. There are close to 200 specific chemicals used in acidization, with at least 28 of them being F-graded hazardous chemicals. Some are used frequently in the range of 100−1000 kg per treatment, such as hydrofluoric acid, xylene, diethylene glycol, and ethyl benzene. Close to 90 more chemicals are identified using non-specific names as trade secrets or reported with no quantity. Unlike hydraulic fracturing the chemical concentrations in acidizing are high, ranging from 6% to 18%, and the waste returns can be highly acidic, in the range of pH 0−3. With this paper it is hoped that acidization becomes part of the larger discussion on concerns with oil exploration and be evaluated by appropriate authorities.

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1. Introduction

Unconventional oil exploration has led to greater energy independence for the USA. It has also raised concerns among the public, NGOs, and policymakers regarding harmful impacts. While researchers have begun exploring the potential impacts of hydraulic fracturing more seriously, impacts from acidizing are not being examined as closely. It is important that acidizing be a bigger part of the discussion to protect the public and environment from potential harm.

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The overall objective of this paper is to identify potential human health risks and environmental hazards associated with acidizing that require further evaluation for responsible decision-making. This paper discusses the differences between three acidizing techniques (acid maintenance, matrix acidization, and acid fracturing) and quantifies the type and amounts of the chemicals used in them. Washington State's Quick Chemical Assessment Tool (QCAT) is used to identify F-graded toxins, which are known carcinogens, mutagens, reproductive toxins, developmental toxins, endocrine disruptors, or high acute toxicity chemicals. QCAT is based on methodology developed by the United States Environmental Protection Agency's (EPA) Design for the Environment program. It looks at various toxicological endpoints (i.e. carcinogenicity, neurotoxicity, etc.) to assign chemicals a hazard grade.

Recently, the California Council on Science and Technology published chemical information for matrix acidization. This chemical information was gathered primarily from the California Department of Conservation's Division of Oil, Gas, and Geothermal Resources (DOGGR). Here, we will use the chemical information from DOGGR as well as the South Coast Air Quality Monitoring District (SCAQMD) to discuss all the acidizing treatment types as well as look at different toxicological endpoints to assign a QCAT hazard grade.

1.1. Description of acidizing techniques

The first use of acids in oil exploration was in 1895, but because of acid attack on metal in the wellbore it was not used much (Putman 1933). The modern-day use of acid in oil stimulation began in the 1930s when it was discovered that corrosion inhibitors could stop acid attack on metal. Commercial use of acids finally began in the 1950s (Williams, Gidley, and Schechter 1979). The idea of using acids for oil stimulation or cleaning a wellbore is an old concept, but the chemicals, volumes, and techniques used in acidizing have evolved.

Acid is used in oil wells as part of three different techniques: acid maintenance, matrix acidization, and acid fracturing. Acid maintenance is a routine procedure used to remove deposits formed on well surfaces, also known as scale. In acid maintenance, operators inject acid solutions at a specific location in the wellbore to react with the scale. The scale is thus cleaned off the surfaces of the wellbore and equipment without any acid penetrating into the formation (Robertson, Chilingarian, and Kumar 1989).

The second technique, matrix acidization, is used to remove formation damage (i.e. blocked oil/gas pathways) around the wellbore and/or increase reservoir rock permeability. Permeability is a characteristic that allows oil and gas to flow through the rock rather than be stuck in pores. Operators inject acid solutions into the well to etch away at the reservoir rock, creating channels for oil and gas to flow through. Matrix acidization does not fracture the formation. Solutions are injected at pressures below the pressure required to fracture the rock, also known as fracture pressure (Robertson, Chilingarian, and Kumar 1989). Matrix acidizing in carbonate formations can nonetheless create small channels or tubes called wormholes that can propagate as much as 6.1 m (20 feet) into the formation, as carbonates are easily dissolved by acids. This is similar to the result of a small hydraulic fracturing treatment (CCST 2014). However, in sandstone, acid dissolution is limited to a much smaller distance of 0.3−0.6 m (1−2 feet) into the formation because the silica

matrix is harder to dissolve. Matrix acidizing in sandstone is therefore primarily used to remove damaging solids that have reduced the near-well permeability of the reservoir. There are some instances of matrix acidizing using HF/HCl reported in the Monterey Formation in California that may have had greater penetration because of the presence of natural fractures (CCST 2014). In California, matrix acidizing is done at depths ranging from 2000 to 11,000 feet. The wells at the lower end of this range come close to drinking water aquifers.

The last acidizing technique is acid fracturing. Acid fracturing, like matrix acidization, uses acids to etch the reservoir. The main difference is the injection rate. Injection rates with pressures below the pressure needed to fracture are termed matrix acidizing, while those above fracture pressure are termed acid fracturing (Mcleod 1986). Acid fracturing is similar to hydraulic fracturing in that a solution is injected into the wellbore at a high pressure to fracture the formation. The difference between acid fracturing and hydraulic fracturing is the composition of the stimulation fluids. Hydraulic fracturing uses a solution consisting of 99.5% water and sand and 0.5% chemicals (US DOE 2009), whereas, as determined from this research, in both matrix acidization and acid fracturing, the concentration of the solution used is 6%−18% chemicals. For acid fracturing to be able to etch channels in the fracture walls, the rock has to be soluble in acid, and the acid should not excessively leak off into the formation without reacting. Thus this technique is mainly used in carbonate formations (Williams, Gidley, and Schechter 1979).

1.2. Acidization fluid make up

Acidizing fluid includes water, acids, and additives. Water is the main solvent and conduit of the chemicals to the wellbore and/or reservoir. Acids are used to dissolve minerals and mobilize mineral grains by decomposing the rock structure. Many other chemicals are added for various purposes that are discussed in the appendix.

The most commonly used acids are listed in Table 1. In California, oil stimulations occur primarily in sandstone and some carbonate formations. Hydrofluoric acid (HF) in combination with other acids is used to dissolve silicates in sandstone. Hydrochloric acid (HCl) and other acids are used to dissolve carbonate minerals, such as limestone. See the supplemental section for more details on which acids are used for different minerals.

Table 1. Acids used in acidizing (Scheiber 2013).

Inorganic	Organic	Mixtures
Hydrochloric acid (HCl)	Acetic acid (CH_3COOH)	Organic acids/HBF_4
Hydrofluoric acid (HF)	Formic acid (HCOOH)	
Mud acid (HCl/HF)	Citric acid ($C_6H_8O_7$)	
Tetrafluoroboric acid (HBF_4)	Biodegradable acids	

1.3. Chemical exposure pathways

There are many different types of chemicals used in acidizing, and the risk of each is dependent on the chemical's exposure, toxicity, fate and transport, transformation, and cumulative and synergistic effects with other chemicals. Many of these factors have not been studied. Here, we focus on the chemicals' human exposure pathways through water.

Table 2. Surface and subsurface chemical release mechanisms into waterways.

Surface	Subsurface
Percolation from unlined pit	Fractured/acid wormhole pathway
Siting of disposal well into aquifer	Deteriorated abandoned well
Inadequately treated wastewater for reuse or disposal	Failure of production or disposal well
Spills, leaks, and accidents	Fault pathway

Table 2 lists the surface and subsurface chemical release mechanisms into waterways from oil stimulation, production, and wastewater management and disposal activities in California. For a diagram of the surface and subsurface release mechanisms see the supplemental section.

The most likely source of water contamination comes from improper handling of wastewater at the surface. Surface release mechanisms include percolation from unlined pits, siting of a disposal well into an aquifer, reuse or disposal of inadequately treated wastewater, and spills, leaks, and accidents. More than half of the wastewater from fractured oil wells in California is disposed of in open, unlined pits and could contaminate groundwater. More than 900 such pits, many without proper permits from the state, lie in the oil fields of the San Joaquin Valley, and some atop usable aquifers (CCST 2015). Wastewater from the surface can also be directly injected into Underground Sources of Drinking Water and "Usable" Water defined under EPA and Bureau of Land Management regulations. The California State Water Board confirmed that at least nine wastewater disposal wells have been injecting waste into aquifers that contain high-quality water protected under federal and state law (Bishop 2014). This becomes a serious issue in drought-stricken places with a high water demand, like the Central Valley of California. Another surface exposure route is reusing or disposing inadequately treated wastewater. In California, about 25% of wastewater was injected into disposal wells or reused for oil/gas extraction. Some wastewater has also been permitted for irrigation in California (CCST 2015). Surface spills, another surface release mechanism, have contaminated both groundwater and surface water. According to the available data between January 2009 and February 2014, 423 surface spills at oil and gas fields in California released nearly 2.8 million gallons of wastewater, or an average of 6500 gallons per incident. Of these, 34 spills released a total of 88,000 gallons of wastewater into California waterways (CCST 2014).

Subsurface release mechanisms include acid wormhole pathways in the rock formation leading to aquifers, fault pathways leading to aquifers, deteriorated abandoned wells leaking into the subsurface, and the failure of production or disposal wells. Modeling work suggests that hydraulic fracturing stimulation fluid contaminating aquifers by connecting pathways is highly unlikely, happening on a 1000−100,000 year scale (Kissinger et al. 2013; Gassiat et al. 2013; Flewelling and Sharma 2014). It is even more unlikely for wormhole pathways to reach an aquifer, because they do not extend very far. However, it becomes a greater threat in California, where unconventional oil stimulation is occurring at relatively shallow depths closer to aquifers with usable water. Matrix acid stimulation has occurred in many fields at depths around 2000 feet (CCST 2014). In both the San Joaquin Valley and the densely populated Los Angeles Basin, oil stimulation has happened at depths less than 1000 feet (CCST 2015). Pathways created by the compromised or failed structural integrity of cement in oil and gas wells and wellbores are considered the most likely potential pathway for groundwater contamination.

2. Methods

2.1. Information sources

Information about the geographical location of acidizing sites in California, volume of water used per treatment, chemical names and chemical abstracts service (CAS) registry numbers, chemical purpose, and chemical amounts were collected, sorted, and analyzed from two California government databases: the Division of Oil, Gas, and Geothermal Resource's (DOGGR) Interim Senate Bill 4 (Interim SB 4) self-reporting portal (DOGGR 2015) and the South Coast Air Quality Monitoring District's (SCAQMD) Rule 1148.2 self-reporting portal (SCAQMD 2015). The data are inclusive of dates from the inception of the Interim SB 4 on 1 January 2014 and SCAQMD's Rule 1148.2 on 5 April 2013 to mid-August 2015.

The data from DOGGR's portal from January 2014 to June 2014 only included information on what operators are required by law to report what they considered as acid fracturing or matrix acidizing; it was not based on a generally applicable regulatory definition. Furthermore, it did not include treatments that used acid concentrations of 7% or less (DOGGR, 2014). After June 2014, operators self-reported matrix acidization or acid fracking based on a uniform regulatory definition with no acid concentration exemption (DOGGR, 2014). As for SCAQMD's data, all chemical information on what operators considered as acidizing was collected. From April 2013 to March 2014 there was no way to determine whether what was reported was acid maintenance, matrix acidizing, or acid fracturing (SCAQMD, 2015), and the chemicals in use during this period could have been representative of any of the techniques. After March 2014, operators reported which acidizing techniques they were using. Information on acid maintenance and matrix acidization was collected from SCAQMD.

There are some clear limitations. Although information is required to be reported, self-reporting reduces transparency; there is no real-time way to validate information or that all information is being reported. Sometimes information is withheld because of trade secrets. Thus, the information may not be representative of consistent and transparent data collection. Furthermore, what was reported to SCAQMD as matrix acidization or acid fracturing should in theory also have been reported to DOGGR because SCAQMD is collecting information for Southern California and DOGGR for the whole state, but there is a lack of expected overlap. The data collected from DOGGR's portal came from only four operators for 100 wells, whereas SCAQMD received information from over 20 different operators for about 500 different wells.

2.2. Hazard assessment

Washington State's Ecology Department's QCAT was used to evaluate hazards associated with acidizing chemicals. The QCAT evaluation is a two-step process that assigns a grade to each chemical.

QCAT examines nine hazard endpoints; carcinogenicity, mutagenicity and genotoxicity, reproductive toxicity, developmental toxicity, endocrine toxicity, acute mammalian toxicity, acute aquatic toxicity, persistence, and bioaccumulation.

Step 1 uses authoritative sources to rank each chemical's hazard endpoints as very high, high, moderate, or low. The authoritative sources for Step 1 are toxicity characteristics

lists and databases generated by internationally recognized authoritative bodies or appropriate government agencies (US NIH, EC-REACH SVHS, IARC, Cal/EPA Prop 65, New Zealand HSNO, Lancet-Grandjean and Landrigan list, etc.). The chemical is ranked in accordance with the strength of the authority and the nature of the classification (Category 1, Priority list, etc.). Once each endpoint is ranked, a grade can be assigned. The grade is assigned on the basis of hazard endpoint-ranking combinations (see supplementary section for the process of assigning an initial grade). For example, a rank of high in carcinogenicity or reproductive toxicity automatically earns the chemical an F-grade.

If the hazard endpoints are found in Step 1 sources they are sufficient for assigning a grade. If however not all the hazard endpoints are found in Step 1 sources, one proceeds to the Step 2 sources. In some instances not all hazard endpoints are needed to assign an F-grade. For example, if a chemical is carcinogenic it is given an F-grade and no information from a Step 2 sources could give it a lower grade. Once the chemical is identified as an F-grade chemical in Step 1 there is no need to look further into Step 2 sources.

Step 2 requires more technical expertize. Its sources could include measured data from publicly available risk assessments and databases such as the Registry of Toxic Effects of Chemical Substances (RTECS) and the Hazardous Substances Data Bank (HSDB), or estimated data from PBT Profiler or other modeling tools. For the purposes of this paper only Step 1 sources were used. All F-graded chemicals have been identified only using Step 1 sources. More F-graded chemicals could be identified through Step 2 sources. Table 3 explains the meanings of the grade levels assigned by the QCAT assessment.

Table 3. Grade levels from the QCAT assessment process.

Grade A	Few concerns (i.e. relatively safe)	Preferable
Grade B	Slight concern	Improvement possible
Grade C	Moderate concern	Use but search for safer alternative
Grade F	High concern	Avoid

Because a QCAT assessment only looks at selected endpoints, chemicals of concerns could be missed during the evaluation process. The QCAT assessment is not as thorough an evaluation of the hazards posed by a chemical as other screening methods, like the GreenScreen® method. However, based on the level of technical expertize required to use it, it is a good starting point to identify hazardous chemicals (Washington State Department of Ecology 2015).

3. Results and discussion

3.1. Acidizing events

Well operators submitted chemical reports to DOGGR for matrix acidizing and acid fracturing only. DOGGR does not require acid maintenance activities to be reported. To SCAQMD, operators submitted chemical reports for acid maintenance and matrix acidizing only. There were no reported instances of acid fracturing to SCAQMD. However, it should be noted that these reports were submitted prior to the actual treatment, and it is not possible to distinguish matrix acidizing from acid fracturing without reviewing well completion reports to determine whether the fracture pressure was exceeded. Operators typically do not distinguish matrix acidizing from acid fracturing. From April 2013 to

March 2014, operators did not distinguish the type of acidizing to SCAQMD. After this time period, operators were required to identify which acidizing technique they would perform. But as stated before the distinction can only be made once an operation is completed. March 2014 onwards there were hundreds of reported instances of acid maintenance and only a handful of cases for matrix acidizing. It is thus likely that the instances of acidizing in the early time period were also mainly acid maintenance cases.

DOGGR acidizing events were primarily in Elk Hills Oil Field in Kern County, California, 40 miles east of Bakersfield, California. They represent 5% of the total unconventional oil stimulation techniques reported to DOGGR; 95% were hydraulic fracturing or listed as other stimulations.

SCAQMD acidizing events were in highly urbanized city centers of Southern California. They represent 65% of the total events reported to SCAQMD. A breakdown of the reported acidizing events can be seen in Table 4. Surprisingly, the SCAQMD matrix acidizing events did not show up in DOGGR's database, though based upon legal jurisdiction they should have. The way local and state authorities define matrix acidizing or an issue of enforcement could explain this discrepancy. There were few acid fracturing events reported to DOGGR and none to SCAQMD. This is probably due to the geology of the region. Acid fracturing is most effective in carbonate formations, whereas most of the oil-bearing formations in California are sandstone. Given the lack of oversight and enforcement and broad range of discretion afforded operators, it is possible that the acidizing events are under-reported.

Table 4. Reported acidizing events in California.

Treatment type	Submitted proposals as of mid-August 2015
SCAQMD acid maintenance	474
SCAQMD acid matrix	6
DOGGR acid matrix	90
DOGGR acid fracturing	10

3.2. Acidizing chemicals

There are many chemicals used in acidizing with data gaps. Many chemicals are listed as trade secrets; others have no toxicological or even basic chemical property information available. As for chemicals with known hazardous endpoints, the amounts used are substantial and create high toxic loads per treatment. The high acidity creates uncertainties as to how chemicals will transform or how much heavy metal will leach out.

This section details the composition and amount of chemicals in acidizing fluids and compares it with hydraulic fracturing. It also details the amounts of F-graded chemicals used in acidizing and what the toxicological load per treatment is – meaning what mass of carcinogens or reproductive toxins, for example, are used per treatment. Finally, it looks at the potential impacts of the acidic fluid.

3.2.1. Composition of acidizing fluids

There are about 200 different chemicals and around 90 chemical families, trade secrets, or chemicals of undefined amount used in acidizing in California. A list of all the chemicals and their amounts reported to DOGGR and SCAQMD, grouped by acidizing technique, is set out in the supplemental materials.

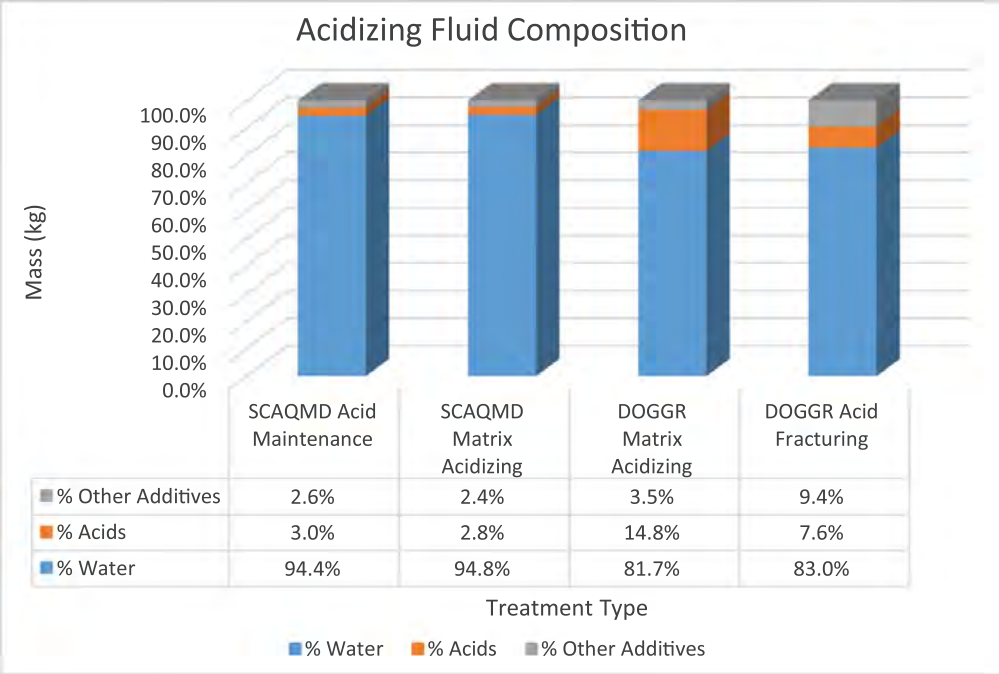

Figure 1. Acidizing fluid composition in California.

Unlike hydraulic fracturing fluid, where chemicals make up only 0.5% of the fluid (US DOE 2009), acidizing chemicals (acids and other chemicals, not including silica in acid fracturing) can make up 17% (acid fracturing), 5%−18% (matrix acidizing), or 6% (acid maintenance) of the fluid. Figure 1 shows a graphical representation of these percentages. Matrix acidizing as reported to DOGGR on average is the most concentrated with at least 81.7% being water and up to 18.3% other chemicals, of which 15% is acid. Acid fracturing as reported to DOGGR is about 8% acid and 9% other additives. In general, acidizing reported to SCAQMD had acids making up 3% of the fluid and other additives around 2.5% of the fluid.

These concentrated fluids have a greater impact than diluted hydraulic fracturing fluid. Microbes are not as effective at breaking down organic chemicals at higher concentrations, making them more persistent in the environment (Kekacs et al. 2015). Furthermore, new research is beginning to show that biocides that are used in unconventional oil stimulation techniques are also not effective at higher concentrations, possibly contributing to bacterial resistance to antibiotics (Kahrilas et al. 2015; Vikram, Bomberger, and Bibby 2015).

Although overall most of the chemicals used in acidizing are similar to the chemicals used in hydraulic fracturing (Stringfellow et al. 2014), those used most frequently in the two operations are different. If we compare the top 20 chemicals in Table 8 of the US EPA Report (2015) (20 most frequently reported additive ingredients in oil disclosures, ranked by frequency of occurrence) to the top 20 frequently used chemicals of acidizing techniques (Tables S4−S7), we see that only 10 of the most frequently used hydraulic fracturing chemicals are those that are used in acidizing treatments (the 10 most frequently used chemicals that are not among the most frequently used chemicals of hydraulic

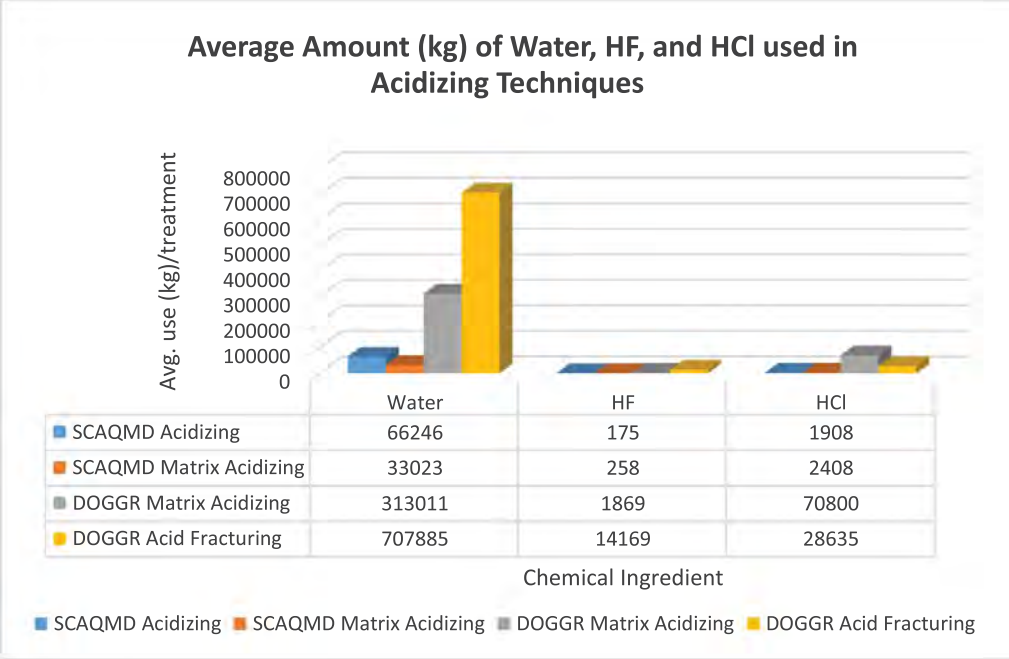

Figure 2. Average amount of water, HF, and HCl used in acidizing techniques.

fracturing are marked with an asterisks in the Tables S4−S7). In other words, 50% of the most frequently used chemicals of acidizing are different from the most frequently used chemicals of hydraulic fracturing, highlighting the need for increased research regarding the effects of acidizing.

Taking a closer look at water and acid use, Figure 2 shows the average amount of water, HF, and HCl used in acidizing.

The treatments reported to DOGGR, whether acid fracturing or matrix acidizing, used more water and acid. Acid fracturing used the most water, close to 700,000 kg/treatment. Acidizing is not as water intensive as hydraulic fracturing, and in general, California oil well stimulations use less water compared to other states. In other states hydraulic fracturing uses 4−12 million kg/well (CCST 2014), whereas in California the average use of water for the various acidizing techniques is between 60,000−700,000 kg/treatment.

Acid fracturing also used the most HF (~14,000 kg/treatment) of the treatments. Matrix acidizing as reported to DOGGR used the most HCl (~71,000 kg/treatment). The acidizing treatments reported to SCAQMD used the least HF (~200 kg/treatment) and HCl (~2300 kg/treatment).

HF is one of the more concerning chemicals, and is used in some of the largest quantities. HF is typically used in a combination with HCl and called mud acid (HF + HCl) to dissolve sandstone as well as remaining drilling mud. In Central California there is a greater use of mud acid. The average concentration by mass percentage (%w/w) of HF and HCl used in mud acid treatments is 0.5%−3% and 4%−15%, respectively (Scheiber 2013). Figure 2 shows that acid fracturing uses the most HF, more than 14,000 kg/treatment, followed by matrix acidizing (reported to DOGGR) at 1870 kg/treatment. All the treatments use more than the minimum reportable quantity of 45.4 kg, as set out by the

Emergency Planning and Notification Act, 40 CFR part 355. Even though acid maintenance uses on average 175 kg/treatment, it is such a common and routine procedure used in wells that the total accumulated load of HF in a region becomes significant. As for matrix acidization, more HF and HCl are used per treatment in Central California (reported to DOGGR) as opposed to Southern California (reported to SCAQMD). This difference could be because of geology or the scale of the activity in these regions. HF is primarily used to dissolve silicates and HCl for carbonates.

HF is of great concern because of its very high acute mammalian toxicity and neurotoxicity. Exposure to fumes or very short-term contact with liquid HF may cause severe and painful burns; it penetrates the skin to cause deep-seated ulceration that may lead to gangrene (National Center for Biotechnology Information 2015). Transport and storage of such large quantities of HF prior to use are serious concerns.

In addition, HF use in wells leaves fluoride behind which can also have detrimental impacts. Fluoride is beneficial in limited quantities for the mineralization of bones and formation of dental enamel. However, excessive fluoride is detrimental; 1.5−4 mg/L results in dental fluorosis, 4−10 mg/L results in skeletal fluorosis, and > 10 mg/L results in crippling fluorosis (Dissanayake 1991). If this excessive fluoride reaches drinking water sources, exposure becomes a serious concern.

The amount of HCl used also creates some concern. Individual reports show that HCl can be up to 270,000 kg/treatment. The %w/w of HCl reported in treatments other than mud acid mixtures is 15%−28%. HCl is corrosive to the eyes, skin, and mucous membranes. Chronic (long-term) occupational exposure to HCl has been reported to cause gastritis, chronic bronchitis, dermatitis, and photosensitization in workers. Prolonged exposure to low concentrations may also cause dental discoloration and erosion (US EPA 1999). Markey et al. (2014) also discuss the health and safety concerns of using HCl in oil drilling, as well as its corrosive impact on flow lines and equipment and environmental effects of the produced HCl.

3.2.2. F-graded chemicals

A main goal of this report was to identify the most toxic acidizing chemicals against a standard criterion. Table 5 lists the QCAT F-graded chemicals that are used in acidizing in California. They are listed in decreasing frequency of use. The primary hazardous toxicological endpoints of these chemicals are noted. These endpoints are detailed with references in the supplemental section.

There are at least 28 F-graded chemicals. The frequency of use and average amount per treatment gives an idea of exposure. However, information about the fate and transport of the chemicals, their transformations, synergistic and cumulative effects, as well as routes of exposure, are vital to understanding their true risk. The most commonly used F-graded chemicals in all acidizing treatments that were used on average in the 100's−1000's kg/treatment are methanol, HF, xylene, and diethylene glycol. These four chemicals are all neurotoxins and in some cases are also developmental or reproductive toxins. Methanol was used in almost all the treatments. HCl, although not listed as an F-rated chemical was also used in most treatments. HF, polyethylene glycol nonylphenyl ether, ethylene glycol, and formaldehyde were used in about half of the treatments. The amount used per treatment and how often a treatment is done are important in understanding the toxicological load put on a certain area.

Table 5. QCAT F-graded chemicals used in acidizing in California (ordered in decreasing frequency of use).

Chemical	CAS #	Maximum (kg)/ treatment	Average (kg)/ treatment)	Frequency of use	Acid maintenance	Matrix acidizing	Acid fracturing	Purpose*	Primary QCAT l ow grade reason**
Methanol	67-56-1	32,062.42	261.86	505	✓	✓	✓	Corrosion inhibitor, non-emulsifier, anti-sludging agents, wetting surfactant, clay stabilizer, solvent	Developmental toxin, neurotoxin, persistent
Hydrofluoric acid	7664-39-3	24,974.71	850.50	290	✓	✓	✓	Acidizing, well stimulation	Acute mammalian toxicity, persistent, neurotoxin
Polyethylene glycol nonylphenyl ether; poly (oxy-1,2-ethandiyl), A-(nonylphenyl)-W-hydroxy	9016-45-9	147.32	30.29	256	✓	✓	✓	Non-emulsifier, surfactant	Developmental toxin, endocrine disruptor, persistent
Ethylene glycol	107-21-1	404.31	11.90	201	✓	✓	✓	Cleaner	Developmental toxin, neurotoxin
Formaldehyde	50-00-0	32.06	0.22	191	✓	✓	✓	Corrosion inhibitor	Carcinogen
Xylene	1330-20-7	11,509.90	361.70	169	✓	✓	✓	Surfactant, solvent, asphaltene dispersant, paraffin inhibitor, demulsifier, cleaner	Reproductive toxin, neurotoxin
Naphthalene	91-20-3	28.08	1.79	169	✓	✓	✓	Surfactant, non-emulsifier, solvent	Carcinogen, acute aquatic toxicity
Ethylbenzene	100-41-4	2578.80	52.40	160	✓	✓	✓	Surfactant, solvent, cleaner	Carcinogen, reproductive toxin, persistent
Cumene	98-82-8	1.39	0.33	146	✓	✓	✓	Demulsifier, surfactant	Carcinogen, neurotoxin
Diethylene glycol	111-46-6	5013.96	1667.81	141	✓	✓	✓	Corrosion inhibitor	Neurotoxin
Ethylene oxide	75-21-8	0.50	0.01	105	✓	✓			Carcinogen, mutagen, developmental toxin, reproductive toxin, neurotoxin, persistent
Silica, amorphous-fumed	7631-86-9	31,895.35	395.51	81	✓	✓	✓	Proppant, friction reducer	Occupational carcinogen, persistent
Crystalline silica	14808-60-7	6769.86	537.70	28	✓	✓	✓	Sand control, proppant	Carcinogen, very high acute mammalian toxicity, persistent
Nitrilotriacetic acid	139-13-9	3206.24	282.67	27	✓	✓		Iron control	Carcinogen, mutagen
Ethanol	64-17-5	857.11	84.12	26	✓	✓	✓	Wellbore cleaner, clay control, iron control	Carcinogen, reproductive toxin, mutagen, persistent
Light aromatic naphtha	64742-95-6	62.23	12.98	21	✓	✓	✓	Non-emulsifier	Carcinogen, mutagen
Cristobalite	14464-46-1	31,895.35	1993.54	16		✓	✓	Proppant, friction reducer	Carcinogen, occupational carcinogen, persistent
Diisopropylnaphthalene/bis (isopropyl) naphthalene	38640-62-9	2.50	1.90	14	✓			Non-emulsifier	Carcinogen, persistent
Toluene	108-88-3	660.11	144.98	13	✓	✓		Solvent, asphaltene dispersant	Reproductive toxin, developmental toxin, persistent

(*continued*)

Table 5. (*Continued*)

Chemical	CAS #	Maximum (kg)/ treatment	Average (kg)/ treatment)	Frequency of use	Acid maintenance	Matrix acidizing	Acid fracturing	Purpose*	Primary QCAT l ow grade reason**
Paraffinic petroleum distillate	64742-55-8	34.51	14.23	12		✓	✓		Carcinogen, persistent
Diethanolamine	111-42-2	27.43	5.30	9		✓	✓	Corrosion inhibitor, surfactant	Carcinogen
Methyl isobutyl ketone	108-10-1	27.43	5.30	9		✓	✓	Solvent	Carcinogen, developmental toxin, neurotoxin
Oxyalkylated alkylphenol; polyethylene glycol mono (branched P-nonylphenyl) ether	127087-87-0	12.23	7.45	6		✓	✓	Surfactant, wellbore cleaner, emulsifier, wetting agent	Developmental toxin, reproductive toxin, endocrine disruptor, persistent
Oxyalkylated alkylphenol; polyethylene glycol nonylphenyl ether; poly (oxy-1,2-ethandiyl), A-(nonylphenyl)-W-hydroxy	26027-38-3	12.23	6.90	5		✓	✓		Developmental toxin, reproductive toxin, endocrine disruptor, persistent
Boric acid (H3B03)	10043-35-3	487.63	299.07	4		✓	✓		Developmental toxin, reproductive toxin, persistent
Cyclotetrasiloxane, 2,2,4,4,6,6,8,8-octamethyl-	556-67-2	0.02	0.02	2	✓			Friction reducer	Reproductive toxin, endocrine disruptor, very persistent
Acrylamide	79-06-1	0.06	0.06	1		✓			Carcinogen, mutagen, developmental toxin, reproductive toxin, neurotoxin
Acrylonitrile	107-13-1	0.0004	0.0004	1			✓		Carcinogen, persistent

*Chemical purposes listed are what the oil operator identifies.
**See the appendix for references for the toxicological endpoints.

The maximum reported use is also listed in Table 5. Some values are far above the average amounts used and may have been mis-reported. Methanol is reported to be used in up to 32,000 kg/treatment, HF up to 25,000 kg/treatment, xylene up to 12,000 kg/treatment, ethylbenzene up to 2600 kg/treatment, diethylene glycol up to 5000 kg/, and silica and cristobalite up to 32,000 kg/treatment. Their various toxicological endpoints can be seen in Table 5.

3.2.3. Toxicological load

In addition to identifying the F-rated chemicals, it is important to understand the toxicological load, or amount of carcinogens or reproductive toxins, for example, per acidizing treatment (see Figure 3).

In acid maintenance there is a high reproductive toxin load from xylene, ethylbenzene, and toluene. The high neurotoxin load is primarily from xylene and HF. Crystalline silica, ethylbenzene, and nitriloacetic acid are the main contributing carcinogens. HF and crystalline silica cause the high acute mammalian toxicity load.

In matrix acidizing as reported to SCAQMD there is a higher reproductive toxin load from xylene and ethylbenzene as well as a neurotoxin load from xylene, methanol, and HF. In matrix acidization reported to DOGGR the high developmental toxin and neurotoxin load are primarily from methanol. HF, xylene, and diethylene glycol also add substantially to the neurotoxin load. The acute mammalian toxicity is primarily from HF. Nitrotriacetic acid is the main carcinogen accounting for the high load.

The acid fracturing treatments have the largest carcinogen load from silica use. The high neurotoxicity and high acute mammalian toxicity in acid fracturing are from HF.

There are between 7000−90,000 kg/treatment of these seven toxicological endpoint chemicals listed in any one acidizing treatment at one time. The actual amounts used in the treatment can be found in the supplemental section (Tables S4−S7). The weighted toxicological impact of these chemicals do not take into account any transformation, or

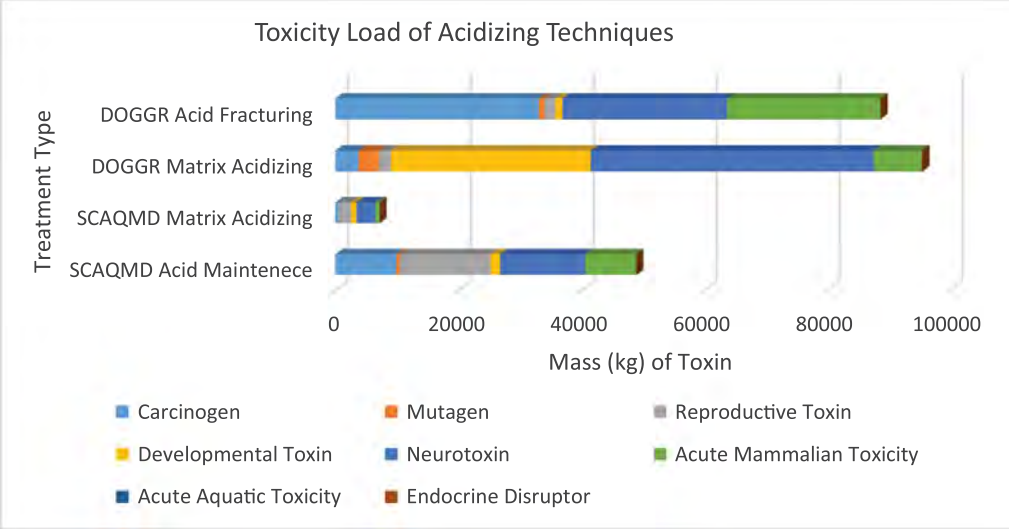

Figure 3. Toxicity load of acidizing techniques in California.

Table 6. Range of pH of matrix acidizing flowback water.

Treatment type	Range of pH of flowback	Time period or volume amount measured over	Reference
HCl/HF (5 diff. treatments)	0.5−3.5 (2.2−3.2)	200 min 500−700 bbl	Schuchart (1995)
15% HCl/1.5% HF	0−3	600 bbl	Gdanski and Peavy (1986)
15% HCl	0.2−5	5 h	Taylor, Nasr-El-Din, and Dajani (1999)

*bbl − oil barrel = 42 US gallon.

synergistic or cumulative impacts. In a highly acidic environment the stability and reactivity of the chemicals are unknown. The potential environmental impact of these chemicals and their byproducts is also unknown. Another problem of acidic solutions is the dissolution and mobilization of naturally occurring heavy metals and other pollutants from the oil-bearing formation, the extent of which is also unknown (CCST 2015).

It should be noted that not only is the acidizing fluid acidic, but the "flowback," in the case of matrix acidization for example, can also be acidic. A few industry reports show that the pH of returning waste is mainly between 0 and 3 for the first few hours (see Table 6). It is unknown how much of the chemicals returns to the surface for acidizing, but recent data submitted to DOGGR by operators show that the volume of recovered fluids collected after matrix acidization is 50%−60% (CCST 2015)

4. Conclusions

The analysis of the present data shows that there have been 474 reported acid maintenance events in Southern California, 96 reported matrix acidization events in Central and Southern California, and 10 reported acid fracturing events in Central California from April 2013 to mid-August 2015. In Southern California, acidization events are occurring in highly urban areas around Los Angeles County. There are about 200 chemicals used in acidization, and 50% of the most commonly used acidizing chemicals are most commonly used hydraulic fracturing treatment chemicals. Unlike hydraulic fracturing, the chemical concentrations in the fluids for acidization are high, ranging from 6% to 18% chemicals, and the waste that returns can also be highly acidic.

The amounts of chemical used per treatment are anywhere between 100's of milligrams and 100,000's of kilograms. Some of the chemicals are known to be of concern for both human health and the environment. An initial hazard assessment was done, and 28 QCAT F-graded chemicals of concern have been identified. It should be noted that close to 90 other chemicals are identified by non-specific names, family classes, or chemicals of undefined amount.

Furthermore, the flowback conditions, pH of the fluid and what chemicals are returning are unknown. The toxicity, the chemical fate and transport, and exposure potential of all these chemicals should be understood, and if a hazard is noted then substitute chemicals should be suggested. Understanding where these chemicals are likely to end up in our environment is critical in predicting how vulnerable populations will be affected. Understanding the toxicity will help us to identify possible impacts we might see in the near to distant future on humans and other living organisms. The stability and reactivity of these chemicals under such strong acid conditions is also unknown. The potential hazard of these chemicals and their byproducts have unknown environmental impacts.

The aim of this paper was to present potential problems of acidizing that need to be investigated so that scientists and legislators can evaluate the environmental impact of acidization. There is a need for the most up-to-date information on what chemicals and quantities are being used and the specific uses of these chemicals. The information in this paper only includes self-reported information, which is limited. There needs to be a transparent way to gather information from industry. Even more important is the need for monitoring and reporting of what is returning as wastewater.

Future research should include identifying appropriate stable indicator chemicals or stable byproduct chemicals from the process that can be monitored in groundwater to track any leakage from wellbores or disposal wells and pits. Possible treatment techniques for chemicals of concern in the wastewater as well as the water remaining in the ground that can contaminate groundwater should also be researched. The feasibility of treatment needs to consider cost effectiveness. If acidization is to be used in oil exploration we need to be aware of the possible impacts and ways to prevent them, including appropriate treatment of residual water at the surface and in the ground.Finally, understanding the current regulatory framework and jurisdiction that is in place to regulate acidization effectively, as well as identifying the gaps that exist, are important in moving forward to safely regulate acidization.

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PLOS ONE

Quantifying Synergy: A Systematic Review of Mixture Toxicity Studies within Environmental Toxicology

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Abstract

Cocktail effects and synergistic interactions of chemicals in mixtures are an area of great concern to both the public and regulatory authorities. The main concern is whether some chemicals can enhance the effect of other chemicals, so that they jointly exert a larger effect than predicted. This phenomenon is called synergy. Here we present a review of the scientific literature on three main groups of environmentally relevant chemical toxicants: pesticides, metal ions and antifouling compounds. The aim of the review is to determine 1) the frequency of synergy, 2) the extent of synergy, 3) whether any particular groups or classes of chemicals tend to induce synergy, and 4) which physiological mechanisms might be responsible for this synergy. Synergy is here defined as mixtures with minimum two-fold difference between observed and predicted effect concentrations using Concentration Addition (CA) as a reference model and including both lethal and sub-lethal endpoints. The results showed that synergy occurred in 7%, 3% and 26% of the 194, 21 and 136 binary pesticide, metal and antifoulants mixtures included in the data compilation on frequency. The difference between observed and predicted effect concentrations was rarely more than 10-fold. For pesticides, synergistic mixtures included cholinesterase inhibitors or azole fungicides in 95% of 69 described cases. Both groups of pesticides are known to interfere with metabolic degradation of other xenobiotics. For the four synergistic metal and 47 synergistic antifoulant mixtures the pattern in terms of chemical groups inducing synergy was less clear. Hypotheses in terms of mechanisms governing these interactions are discussed. It was concluded that true synergistic interactions between chemicals are rare and often occur at high concentrations. Addressing the cumulative rather than synergistic effect of co-occurring chemicals, using standard models as CA, is therefore regarded as the most important step in the risk assessment of chemical cocktails.

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Introduction

Background

Cocktail effects and synergistic interactions of chemicals in mixtures are an area of great concern to both the public [1,2] and regulatory authorities in the US and Europe ([3] and references therein). There are two general aspects underlying this concern: The first is the uncertainty as to whether we are monitoring and regulating the most harmful chemicals? The second concerns whether the chemicals we regulate on a single compound basis, and deem ''safe'', potentiate or are being potentiated by other chemicals so that they jointly exert a larger effect than predicted? The latter is called synergy, and is one of the factors that create uncertainty around models proposed for the implementation in chemical risk assessment of mixtures. For those legislations where mixtures are considered, which are few, dose- or concentration additivity is proposed as the default model [3]. But are synergistic interactions really an area that should concern us? Earlier reviews have shown that synergistic interactions, at least within pesticide mixtures and realistic low-dose chemical mixtures in mammals, are a rather rare phenomenon, constituting approximately 5% of the tested mixture combinations [4–7]. This percentage is rather low given the fact that experiments are often designed to search for synergistic interactions, thereby biasing the databases towards synergistic interactions. If, however, these 5% are combinations that often co-occur in humans and the environment, they might nonetheless be of quantitative importance. Hence, if we could identify the groups of chemicals that are likely to induce synergistic interactions, special precautions could be taken in the risk assessment of these chemicals. Identifying the potential synergists would reduce the uncertainty of using the models proposed for risk assessment of mixtures of the remaining 95% of antagonistic or non-interacting chemicals [3].

The aim of this review is therefore to define which groups of chemicals are involved in well documented synergistic interactions, and if possible, to identify the mechanisms behind their synergistic effects. This will be done within three large groups of chemicals that often co-occur in the environment at measurable concentrations: The first group consists of pesticides, which is probably the most well studied chemical group within ecotoxicological mixtures studies. This is not only due to the use of chemical mixtures in pesticide formulations and tank mixtures and the resulting co-occurance in agricultural areas, but just as much because of the in depth knowledge of their physiological mode of action. This makes them ideal candidates for testing mixture models based on chemical mode of action and understanding the physiological mechanisms behind possible interactions [8,9]. The second group of chemicals are metals. Metals typically co-occur in potentially

toxic concentration in relation to mining, smelting and other industrial activities and a substantial body of literature on metal mixtures is available [10]. The last group are antifouling biocides, which consist both of traditional organic biocides, organo-metals and metal ions [11], making this group a mixture of the two above thereby opening the possibility of finding other synergistic mechanisms. Antifoulants co-occur in harbour areas and marine and freshwater areas with substantial boat traffic [12,13].

Chemical mixtures from waste water treatment plants, oil spills, industrial effluents and other sources yielding very complex mixtures have not been included for two reasons: The first is that they are often chemically very poorly described; hence, we do often not know which chemicals cause the majority of the toxicity [14,15]. The second is that the probability for severe interactions decrease when the number of pollutants adding to the joint toxicity increase [16,17], hence, severe interactions are more likely to occur when a few chemicals dominate the overall toxicity, as is more often seen for e.g. pesticide pollution, in comparison to effluent pollution [18].

The Definition of Synergy

Defining synergy as two or more chemicals exerting a larger effect than predicted implies that we can predict joint effects of chemicals under certain assumptions. The aim of being able to do so, has been a research topic for more than a century [19], and the two major concepts underlying all valid assessments of joint chemical effects were framed already in the first part of the twentieth century by Loewe and Muischnek (1926) and Bliss (1939), respectively [20,21]. Loewe and Muischnek (1926) based their concept on the assumption that all chemicals in a mixture acted on the same biological target site and therefore could be viewed as being dilutions of each other, each having a different chemical potency. Hence, if the chemical potency of chemical A and B in a binary mixture was based on the Effect Concentration (EC) of each chemical causing x% effect on any endpoint in a test-system ($1/EC_{xA}$ and $1/EC_{xB}$), then the sum of the concentration of chemicals (c_A and c_B) multiplied with their respective potency in a mixture provoking x% effect would be equal to 1 [20]:

$$\frac{c_A}{EC_xA} + \frac{c_B}{EC_xB} = 1 \qquad (1)$$

The concept has been re-invented several times since 1926 and has received many names such as Loewe Additivity, Dose Addition, The Additive Dose Model or Concentration Addition, depending on whether it has been used within pharmaceutical, agricultural, toxicological or ecotoxicological sciences [22]. In this review we will use the term Concentration Addition (CA). Bliss (1939) worked with test-systems where mortality was the endpoint, and added another way of looking at mixtures, in the cases where the tested chemicals obviously did not affect the organisms through a similar molecular target. Different target sites were by Bliss defined by their concentration-response curves having different shapes [21]. Bliss viewed death by a chemical as a stochastic event. The probability of surviving or dying due to exposure to several chemicals acting on independent targets in the organism could therefore be calculated based on probabilities of surviving or dying from exposure to the individual chemicals [21]. Hence, the probability of surviving two independently acting chemicals (R_{mix}) would be equal to the probability of surviving the first chemical (R_1) multiplied by the probability of surviving the second chemical (R_2). Or, if assessing the probability of dying from two independently acting chemicals (E_{mix}), this is equal to the probability of dying from the first chemical (E_1) plus the probability of dying from the second chemical (E_2), minus the probability of dying from both chemicals ($E_1 \times E_2$) [21].

$$E_{mix} = E_A + E_B - E_A E_B \qquad (2)$$

This concept has likewise been re-invented several times and has been named Bliss Independence, Response Multiplication, Response Addition, Effect Addition, Independent Action a.o. depending on the inventor and context [22]. In this review we will use the term Independent Action (IA). Both concepts can be extended to an infinite number of chemicals and can be used to predict mixture toxicity effects of all mixture ratios and effect levels, providing that entire dose- or concentration response relationships for the single chemicals in the desired test-system are available. Often such data are not available and reduced approaches must be used. A recent review of mixture models and their uses can be found in Cedergreen et al. (2013) [22]. How they are proposed to be used in different chemical legislation is reviewed by Backhaus and Faust (2010) [3]. Common for both concepts is also the assumption that the chemicals do not interact chemically or affect the toxicity of each other [20,21]. If the chemicals do interact, the joint effects might deviate from the predictions resulting either in the before mentioned synergistic effects or in antagonistic effects, which are defined as smaller effects than predicted [22].

Synergy can therefore be defined in relation to two basic concepts: CA and IA. Empirical evidence, however, shows that even mixture toxicity of dissimilarly acting compounds can be described with a high level of accuracy with CA, as well as with IA, despite their different underlying assumptions [6,23]. CA generally generate slightly more conservative predictions (predicting larger effects than IA), and as databases on chemicals often only provides EC_x data or No Observable Effect Concentrations (NOECs) or Lowest Observable Effect Concentrations (LOECs) which only makes CA predictions possible and not IA, CA is most often the recommended model for risk assessment purposes [3]. In this review, synergy is therefore defined in relation to CA predictions.

Experimental data are always determined with variance. For mixture studies this applies both to the toxicity data of the individual compounds used to make the model prediction, and to the tested mixture toxicity data. The consequence of this is that small deviations from the reference models can be difficult to detect statistically and repeat experimentally [24]. Biologically significant and reproducible synergy is therefore here defined as a more than two fold deviation from CA, as was also proposed by Belden et al (2007) [5]. That is, the concentration predicted to yield a certain effect is more than twice the concentration actually observed giving the proposed effect [5]. Belden calls the ratio of predicted versus observed effect concentrations for the Model Deviation Ratio (MDR) [5]. Many of the mixtures showing MDRs slightly below two, most likely also include true synergists. But to exclude false positives and to focus on combinations where the size of the synergistic interactions might be of quantitative importance, we have chosen to set the MDR limit defining synergy at two.

Materials and Methods

Identification of Experiments

A flow chart of the record selection for each of the three toxicant groups is presented in Figure 1. To evaluate the frequency of chemicals, chemical mixtures and species groups involved in synergistic (MDR>2), additive (0.5≤MDR≤2) and antagonistic

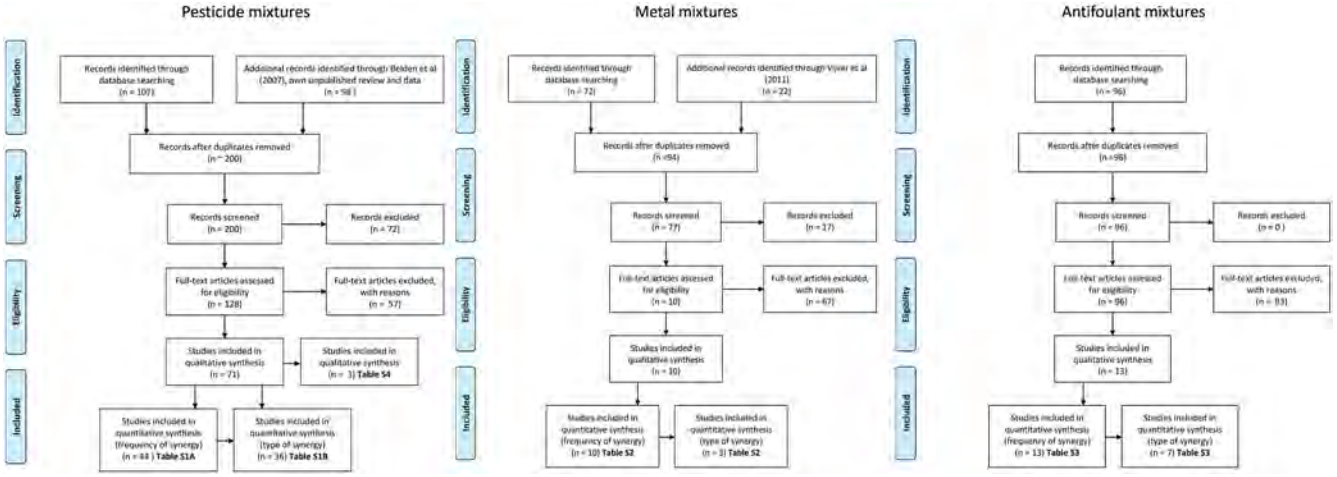

Figure 1. PRISMA 2009 Flow Diagram [90]**.** A flow diagram depicting the process of selection of records used in the review for the three main groups of toxicants: Pesticides, metals and antifoulants. Data selection has, for pesticides and metals, been built on previous reviews and data compilations, given in the top right text-box, supplemented with database searched using ISI Web of Science. Search criteria and criteria for selecting eligible records are given in the Material and Methods section. For each toxicant the search resulted in two types of databases: One to determine the frequency of synergy in a randomly selected number of mixtures studies, and another focussing only on defined synergistic mixtures. It should be noted that many records contain data on several independent mixtures studies; hence the number of records given in the figure does not match the number of selected studies reported in the results section. References to tables in supporting material giving the raw data on specific chemical mixtures, test species, endpoint and timecourse of the experiment, and the record providing the information are given in the figure.
doi:10.1371/journal.pone.0096580.g001

(MDR<0.5) mixture experiments, the database of Belden et al. (2007) was used for the pesticides, the one of Vijver et al (2011) was used as a starting point for the metals, while our own data-collection was used for the antifoulants (Figure 1, Supporting information: Table 1A, 2 and 3).

For the pesticide mixtures, Belden et al (2007 and our own database on synergistic interactions was expanded with more recent studies screening the database ISI Web of Science using the search words ''pesticide*'', ''mixture*'' and ''synerg*'' in the period 2008–2013. For the metals, the review by Vijver et al (2011) [10] was supplemented by newer studies using ISI Web of Science and the search words ''metal*'', ''mixture*'', ''synerg*'' and ''toxic*'' for the period 2009–2013. The antifoulant mixture compilation using ISI Web of Science and the search words ''antifoul*'' and ''mixture*'' for the time period 1990–2013 to be able to detect the frequency of synergy in a similar way as had been done in the study by Belden et al. (2007).

Only studies complying with the criteria developed by Belden et al (2007) were used: Mixture studies should be conducted using only pure substances. Hence, studies using formulated pesticide or formulated antifouling biocides were excluded, as the formulation products could affect the results. Studies using metals in the form of nano-particles were likewise excluded. To avoid biasing the database with similar experiments, duplicated experiments using the same mixture and species presented in the same manuscript were entered in the database as one study, but giving the MDRs of each individual replicate. If multiple mixture ratios were tested in the same experiment, the MDR from the mixture ratio closest to the ratio where both chemicals contributed equally to the toxicity (equipotent ratio) was used in the cases of isobole designs, where several mixture ratios were tested. Otherwise the numerically larger MDR was used. Finally, the experiment had to be conducted in a way that an MDR could be calculated. That is, comparable *ECx* values or Toxic Units ($1/EC_x$) from individual compounds and their mixtures should be available either directly or from reading off graphs. From each study, the following information was collected: The chemicals involved, the species tested, the higher taxonomic group of the species, monitored endpoint and duration of the toxicity test, and the original reference where the raw data were reported. Studies on species communities were not included.

It should be noted that the published data does not represent a random selection of chemical mixtures tested on representative ecological species, but rather represent mixtures selected because of co-occurrence or suspicion of synergy tested on standard laboratory species. The choice of chemicals biases the database towards detecting synergies, while the choice of robust laboratory organisms, on the other hand, might give conservative estimates on synergies as they might not represent the most susceptible species.

All data treatments were done in excel.

Results

The Frequency of Synergy

Figure 1 presents the selection process of record for the study. Several of the records reported more than one mixture toxicity experiment. In the following the individual mixture toxicity experiments will be discussed. The records from where data has been retrieved can be found in the tables S1–S4 in File S1 in the supporting information. A PRISMA Checklist for reviews is given in Checklist S1.

Pesticide mixtures. The database of Belden et al (2007) provided data on 207 pesticide mixtures of which 194 were binary and another 13 consisted of more than two pesticides [5].

Metal mixtures. Evaluating the meta-analysis of Vijver et al (2011) on metal mixtures according to the criteria set by Belden et al (2007), reduced the number of usable studies from 22 to 6 studies reporting 10 experiments where MDR could be calculated and another 7 experiments, where data shown on graphs could be evaluated as being over or under-predicted by CA (Table S2 in File S1). Of the remaining 15 studies, five studies only allowed IA predictions and 10 studies reported metal tissue accumulations, but not effects. Since there is not always a straight forward

Table 1. The overall group, name and proposed Modes of Action (MoA) of the antifouling compounds.

Group	Name	IUPAC name	Mode of Action
Photosystem II inhibitors	Atrazin	6-chloro-N^2-ethyl-N^4-isopropyl-1,3,5-triazine-2,4-diamine	Inhibits the electron transport in photosystem II
	Irgarol1051	2-tert-butylamino)-4-(cyclopropylamino)-6-(methylthio)-1,3,5-triazine	Inhibits the electron transport in photosystem II
	Seanine211	4,5-dichloro-2-n-octyl-4-isothiazoline-3-one	Inhibits the electron transport in photosystem II
	Diuron	3-(3,4.dichlorophenyl)-1,1-dimethylurea	Inhibits the electron transport in photosystem II
Metals and organometals	Cd	Cadmium ion	General toxicant, interacts with enzymes[a]
	Cu	Copper ion	General toxicant, interacts with enzymes[a]
	CuPT	Copper 2-pyridinethiol-1-oxide	General toxicant, interacts with enzymes[a]
	Zn	Zink ion	General toxicant, interacts with enzymes[a]
	ZnPT	Zinc 2-pyridinethiol-oxide	General toxicant, interacts with enzymes[a]
	Ziram	Zinc bis(N,N′-dimethyl)-dithiocarbamate	Dimethyldithiocarbamate fungicide with Zn. Inhibitor of enzymes containing copper ions or sulfhydryl groups, including P450 monooxygenases of the CYP 2A6 group[b]
	TBT	tri-butyl-tin-chloride	PSII inhibitor (with tin), endocrine disruptor[c]
Fungicides	Chlorothalonil	Tetrachloroisophthalonitrile	Conjugation with, and depletion of, thiols (particularly glutathione) from germinating fungal cells, leading to disruption of glycolysis and energy production, fungistasis and fungicidal action.
	Dichlofluanid	*N*-dichlorofluoromethylthio-*N*′,*N*′-dimethyl-*N*-phenylsulfamide	Multi-site mode of action, non-specific thiol reactant, inhibiting respiration.
	IPBC	3-iodo-2-propynyl butylcarbamate	AChE inhibitor and fungicide and bactericide[d]
	PTPB	Pyridine triphenylboron	Fungicide[d]
	TCMTB	2-thio cyano methyl thio benzothiazole	Fungicide, Inhibitor of mitochondrial electron transport[c]
	Tolylfluanid	*N*-dichlorofluoromethylthio-*N*′,*N*′-dimethyl-*N*-*p*-tolylsulfamide	Multi-site mode of action, non-specific thiol reactant, inhibiting respiration.

[a]Altenburger, 2011 [88].
[b]Walker, 2009 [49].
[c]Fernandez-Alba et al, 2002 [89].
[d]Zhou et al, 2006 [40].
Particularly for the fungicides, which have multiple and often undefined modes of action, different target sites are given in different references. For herbicides and fungicides used as pesticides we use the definition of Tomlin 2002 [29]. For the remaining compounds, the source of the MoA are given as footnotes.
doi:10.1371/journal.pone.0096580.t001

correlation between tissue accumulation and toxic effect [25,26] these were disregarded. A recent paper of Xu et al (2011) [27] added another 11 metal mixtures where MDR-values could be calculated, making it a total of 28 mixtures from 8 studies tested on 7 species. Of these, 21 mixtures were binary while the remaining 7 mixtures consisted of more than two metals.

Antifoulants mixtures. For antifoulants 136 mixtures where MDR-values could be calculated were found. These were presented in 14 studies comprising mixtures of 20 chemicals tested on 15 different species (Table S3 in File S1). There were 103 binary mixtures and 33 mixtures with more than two chemicals. The frequencies of synergy in the binary mixtures were 7%, 3% and 26% for pesticides, metals and antifoulants, respectively, while 88%, 86% and 64% was within two fold of the CA prediction (Figure 2). For the 13 pesticide mixtures where more than two chemicals were included, only one was synergistic [5], while for the 33 antifoulant mixtures with more than two chemicals 61% showed severe synergy (Table S3 in File S1).

Types of Synergy

Pesticide mixtures. In addition to Belden et al (2007) [5] and the review by Cedergreen et al (2008) [28] another 84 papers were reviewed for synergy where the MDR ratios were >2. This resulted in a database on synergistic interactions including 73 cases of synergy from both Belden et al (2007) and the data search compiled from 36 studies. These studies tested the effect of combinations of 54 pesticides on 27 different species. Of all the mixture combinations, 69 were binary mixtures while the remaining four mixtures consisted of combinations of three or five organophosphate insecticides or eight chloroacetamide herbicide safeners (Table S1B in File S1). Dividing the pesticides into groups with common modes of action according to Tomlin

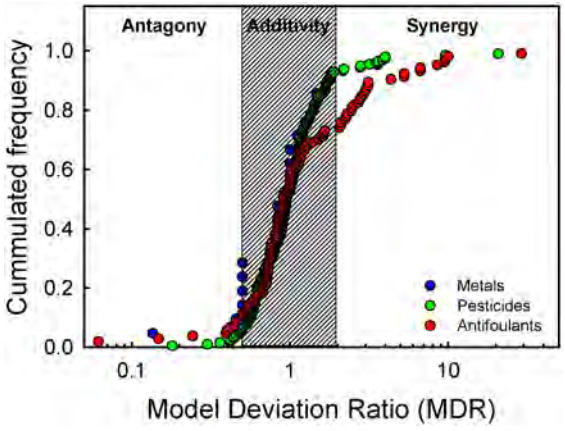

Figure 2. Cummulated frequency of Model Deviation Ratios. Cummulated frequency of Model Deviation Ratios. (MDR) of binary mixtures of pesticides ($n = 195$), metals ($n = 20$), and antifoulants ($n = 103$). The hatched interval where $0.5 \leq MDR \leq 2$ defines the mixtures that deviates less than two-fold from a Concentration Addition predictions. Mixtures having MDR values<0.5 are termed antagonistic, while mixtures with MDR values>2 are synergistic.
doi:10.1371/journal.pone.0096580.g002

(2002) [29] showed that particularly five groups of pesticides were overrepresented in the synergistic mixtures. These were the organophosphate and carbamate insecticides (Cholinesterase inhibitors), azole fungicides (Ergosterol biosynthesis inhibitors), triazine herbicides (Photosystem II inhibitors) and pyrethroid insecticides (interferes with sodium channels in nerve cells) (Figure 3A). Grouping the cholinesterase inhibitors together and looking at which of the binary combinations of the above pesticide groups induced synergy in auto-trophic organisms (plants and algae) and hetero-trophic organisms (microorganisms and animals) showed no cases of synergy within the autotrophic organisms (Figure 3B). In the group of hetero-trophic organisms 69 of the 73 synergistic mixtures (95%) contained either cholinesterase inhibitors (organophosphates or carbamates) or azole fungicides (Figure 3C). The remaining four mixtures were the before mentioned mixture of 8 herbicide safeners, a mixture of a pyrethroid with an organochloride insecticide, a pyrethroid insecticide and a piperidine fungicide and a photosystem II (PSII) inhibiting herbicide and a growth regulator (Table S1B in File S1). Of the 69 binary mixtures 76% contained a cholinesterase inhibitor and another 24% an azole fungicide (Figure 3C). The triazines only entered in synergistic mixtures together with either chlorpyriphos, diazinon, malathion, methidathion, methyl-parathion, which belong to the phosphorothioate and phosphorodithioates class of organophosphates, or trichlorfon, a phosphate class organophosphate. Pyrethroids, on the other hand, only entered in synergistic mixtures together with azole fungicides.

An evaluation of which types of the pesticides from the review of Belden et al (2007) were dominant in the antagonistic mixtures and those conforming to CA, showed that cholinesterase inhibitors and azole fungicides made up 29% of the antagonistic mixtures and 48% of the mixtures conforming to CA (Figure 3B and C), which is considerably less than the 95% of the synergistic mixtures. Hence, though these modes of action were present in all types of mixtures, they were clearly overrepresented in the mixtures displaying synergistic interactions. The triazines occurred in 1% of the antagonistic mixtures, 22% of the concentration additive mixtures and in 12% of the synergistic mixtures. Hence, triazines did not seem to occur particularly frequently in the synergistic mixtures, and when they did, only in mixtures with the before

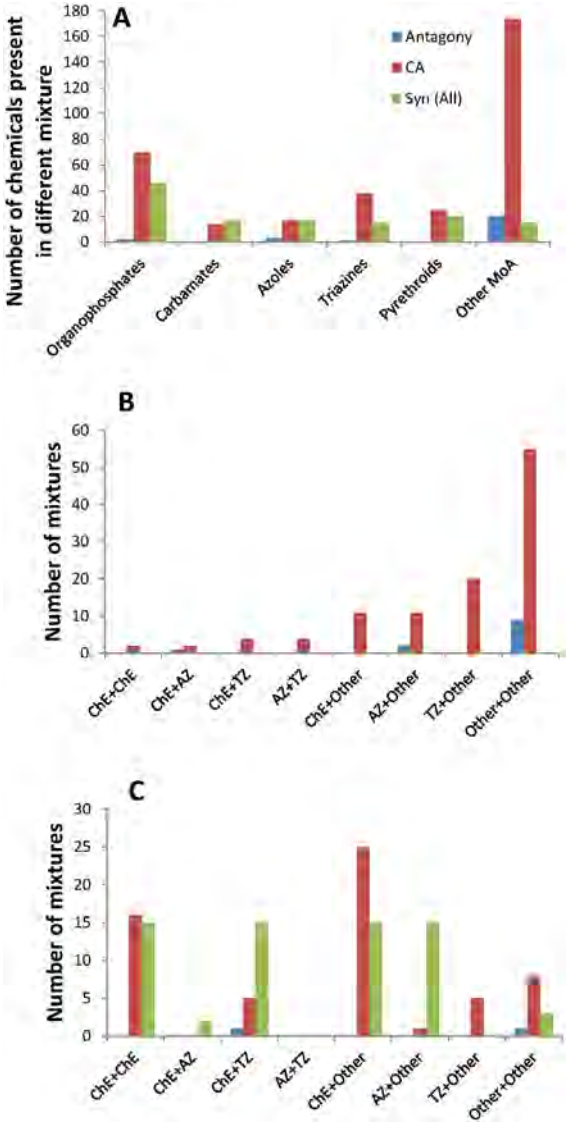

Figure 3. Frequency of pesticide antagony, additivity and synergy. Figure 2A shows the number of times a pesticide belonging to the group of organophosphates, carbamates, azoles, triazines, pyrethroids or some other Mode of Action (other MoA) occur in a binary mixture resulting in antagony (blue bars), concentration additivity (CA) (red bars) or synergy (green bars). In figure B and C, the number of binary combinations of cholinesterase inhibitors (ChE) (The organophosphates and carbamates), azoles (AZ), triazines (TZ) and other Modes of Action (Other) resulting in either antagony, concentration additivity or synergy are shown for mixtures tested on B) autotropic organisms (plants and algae, $n = 120$) or C) heterotrophic organisms (microorganisms and animals, $n = 128$).
doi:10.1371/journal.pone.0096580.g003

mentioned organophosphates. The 19 triazine mixtures with an MDR<1 were dominated by Auxin transport inhibitors, branched chain- and aromatic amino acid synthesis inhibitors, while the 19 triazine mixtures with MDR values between 1 and 2 were dominated by organophosphates, PSII inhibitors and cell division inhibiting herbicides. All 22 additive mixtures including pyrethroids, were mixtures with organophosphates, carbamates or other pyrethroids (Table S1A in File S1).

Metal mixtures. Going through the 55 selected potential papers found on ISI Web-of-Science using the key-words given

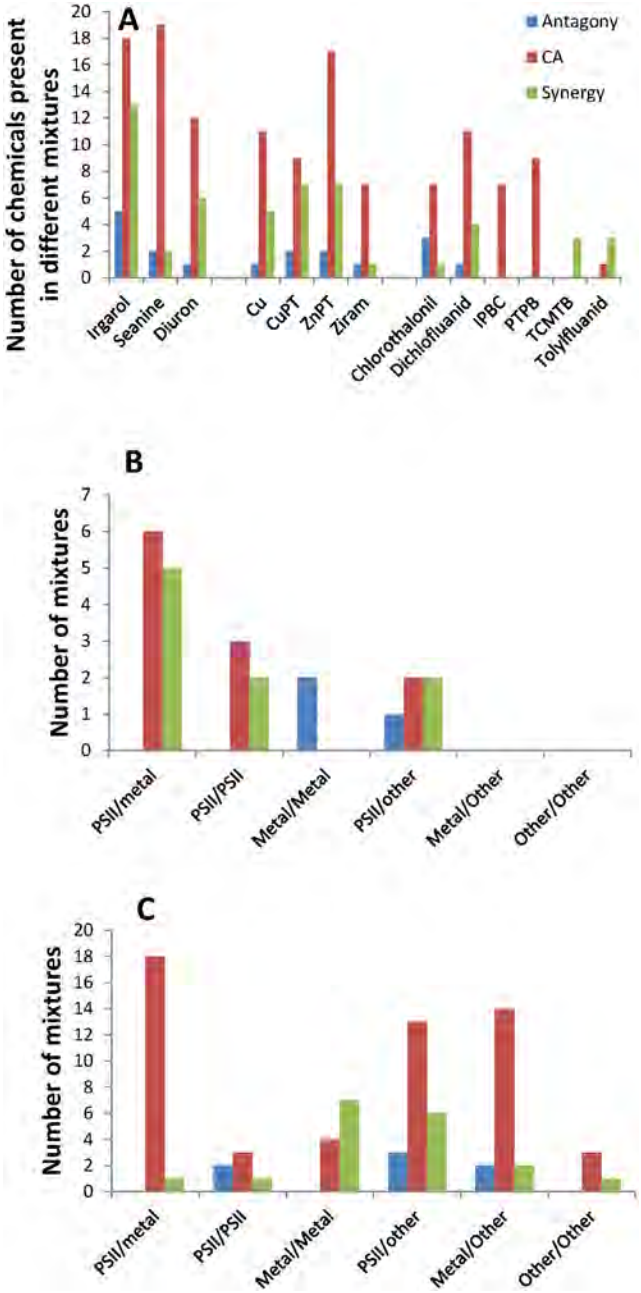

Figure 4. Frequency of antifoulant antagony, additivity and synergy. Figure 3A shows the number of times each of the antifoulants occur in a binary mixture resulting in antagony (blue bars), concentration additivity (CA) (red bars) or synergy (green bars). Antifoulants occurring in less than 1% of the mixtures were excluded. In figure B and C, the number of binary combinations of photosystem II herbicides (PSII) metal ions or metal containing compounds (Metal) and other organic compounds (Other) resulting in either antagony, concentration additivity or synergy are shown for mixtures tested on B) auto-tropic organisms (plants and algae, $n = 23$) or C) heterotrophic organisms (microorganisms and animals, $n = 80$).
doi:10.1371/journal.pone.0096580.g004

above only revealed two additional studies with three experiments where MDR>2 could be estimated from figures (Table S2 in File S1). Hence, despite the large numbers of studies made on metal mixtures, well documented severe synergistic metal-metal interactions seem to be rare. The four binary mixtures giving synergy were, Cd+Zn, Cu+Zn, Cu+Cd and Cd+As tested on the shrimp *Penaeus setiferus*, the fish *Gobiocypris rarus* and the water-flee *Daphnia magna* (Table S2 in File S1).

Antifoulants mixtures. In the antifoulants database (Table S3 in File S1), we found 47 cases of synergy from 8 studies, testing the effect of mixtures of 12 chemicals on 9 organisms. Another 7 chemicals were tested that did not occur in any of the synergistic mixtures. The antifoulants were more difficult to categorise according to physiological mode of action compared to the pesticides, as this information is not required for registration. While pesticides are often developed to act physiologically very specifically in specific target organisms, antifoulants are selected to be toxic to the wide range of organisms settling on ship hulls. Hence, their physiological mode of action is more likely to be general, targeting physiological pathways important for a broad range of species. Hence, the analysis of the frequency of chemicals in synergistic, additive and antagonistic mixtures were done on the individual chemicals roughly divided into three groups: Herbicides (2,4-D, atrazine, irgarol 1051, Seanine 211, and diuron), metals and metal containing organic compounds (Cd, Cu, Cu Pyrethrione (PT), Zn, ZnPT, Ziram and Tributhyltin (TBT)), and other organic compounds (chlorothalonil, dichlorfluanid, 3-iodo-2-propynyl butylcarbamate (IPBC), pyridine triphenylboron (PTPB), 2-thio cyano methyl thio benzothiazole (TCMTB) and tolylfluanid). Chemical class and proposed modes of action are given in Table 1. For the 103 binary mixtures the frequency of synergy was markedly higher than the frequency of antagony for mixtures containing either irgarol or diuron, Cu, CuPT or ZnPT, TCMTB, dichlorofluanid or tolyfluanid (Mixtures including Cd, Zn, or TBT were excluded in this analysis as they were included in <3 binary mixtures each) (Figure 4A). Analysing the frequency of binary mixtures combined of the above defined three overall groups for the 23 binary studies on plants and algae and the 80 studies on animals and microorganisms separately, showed that all synergistic mixtures tested on plants or algae contained a PSII inhibiting herbicide either in combination with another PSII inhibiting herbicide, or metal or an organic antifoulant (Figure 4B). This is contrary to the pesticide study, where no synergy was found in studies on auto-trophic organisms at all (Figure 3B and C, Table S1B in File S1). For the autotrophic organisms the PSII inhibitors were present in slightly more additive than synergistic mixtures (Figure 4B). For the group of heterothrophs, PSII inhibiting herbicides also caused synergy, particularly together with dichlorfluanid, tolyfluanid and TCMTB (Figure 4C, Table S3 in File S1). Combinations of two metal containing compounds induced synergy in seven of 11 cases for this group (64%). Hence, contrary to mixtures of metal ions (Table S2 in File S1), mixtures of organically bound metals seem to be much more potent in inducing synergy.

Of the 23 ternary mixtures of antifoulants and the ten quarternary mixtures, four mixtures were antagonistic, nine additive and the remaining 20 mixtures had an MDR>2 (Table S2 in File S1). The frequency of occurrence of the different antifoulants in antagonistic, additive and synergistic mixtures is shown in figure 5, confirming that particularly irgarol, Seanine, CuPT, dichlofluanid and tolylfluanid often occur in synergistic mixtures.

Synergistic mixtures of metals and organic compounds. In the search for synergistic mixtures, some mixtures showed up that could not be categorised as either pesticides or metals, as they contained both. Synergistic interactions between metals and pesticides seemed to be quite frequent compared to synergistic mixtures of metals alone, but since a comprehensive database on mixture experiments of metals and

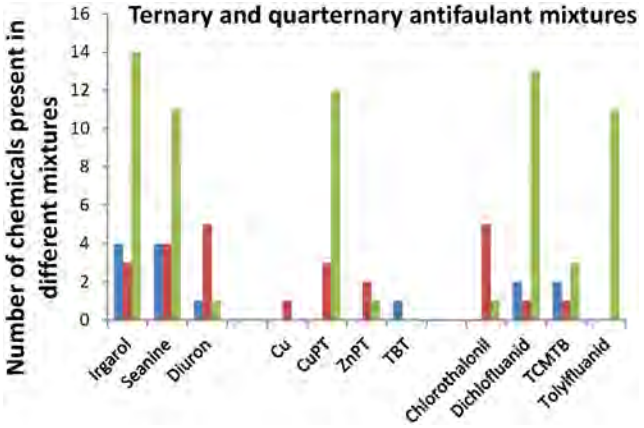

Figure 5. Frequency of antifoulant interactions in ternary and quaternary mixtures. The number of times each of the antifoulants occur in a ternary ($n = 23$) or quaternary ($n = 10$) mixture resulting in antagony (blue bars), concentration additivity (CA) (red bars) or synergy (green bars).
doi:10.1371/journal.pone.0096580.g005

pesticides has not been made, this cannot be tested. Table S4 in File S1 in the Supplementary material show 11 mixtures of metals and pesticides from three studies, of which eight had a MDR value >2.

Discussion

Which are the Chemicals Causing Synergy?

The review showed that for pesticides, the combinations causing synergy were not random but included either cholinesterase inhibitors or azole fungicides in 95% of the described cases. The proposed mechanisms behind these synergies are relatively well investigated, as discussed below. The synergy frequency for metal ion mixtures was very low, hence no general conclusion in terms of which compounds caused synergy could be made. When metal ion synergy occurred, it was in the mg L^{-1} concentration range for three of the four cases [30,31]. These concentrations are high, compared to the concentrations normally found in metal polluted waters being in the lower ng to μg L^{-1} range [32,33]. For the antifouling compounds synergistic interactions were also related to specific chemical groups, though more synergistic combinations of different chemical groups were involved than seen for the pesticides (Figure 3, 4). The high frequency of synergistic interactions observed for the antifoulants, particularly in the mixtures with more than two active ingredients, is most likely due to the selection for compounds able to induce synergy in antifouling products, which most often are composed of more than one active ingredient [34]. The mechanisms behind the synergistic interactions of the antifoulants are, contrary to what is seen for the pesticides, rarely investigated. In the following, the proposed mechanisms behind the synergistic interactions of pesticide, metal ions and antifouling mixtures are discussed.

Mechanisms Causing Synergistic Interactions

Interactions between chemicals can basically affect six processes that are important for the resultant toxicity of a chemical towards an organism: bioavailability, uptake, internal transportation, metabolization, binding at the target site and excretion. The synergistic interactions identified in the present study are most likely caused by interactions around one or more of these processes. In the following, known mechanisms behind the identified synergistic interactions will be discussed in terms of which processes are most likely affected by the interactions.

Bioavailability. Interactions between chemicals can take place outside the organism, with one chemical affecting the availability of the other. This is commonly seen for metal ions, where ion speciation and competition for binding sites to organic matter in soil, sediments and the water phase can change free ion availability and composition [35–37]. If a less toxic ion replaces a bound or chelated ion with a higher toxicity, this will lead to apparent synergistic interactions, if the toxicities are estimated based on total metal concentrations rather than bioavailable concentrations. These types of interactions, however, most often occur when binding sites are limited. Hence, either the metal ion concentrations are high, or the binding site density low, as would for example be the case with ions in water with low concentrations of dissolved organic matter or mineral ions as calcium carbonate and other salts [37]. None of the four metal-metal ion synergies found in this study (Table S4 in File S1) allow for an assessment of whether the synergistic interactions occurred due to changed ion availability, as only total metal concentrations were given. Changes in speciation outside the organism as a cause of synergistic interaction has, however, been well documented for mixtures of pyrithione antifoulants [38]. When ZnPT and Cu ions are mixed together the more toxic CuPT complex is formed, making the mixture more toxic than predicted from the toxicities of ZnPT and Cu alone [38–40]. As the affinity of pyrithione for Cu is higher than for Zn, then the equilibrium between the metal-pyrithione complexes and free pyrithione will be shifted in favour of CuPT [41]. If there is a metal ion surplus in the ZnPT and CuPT synergistic mixture observed by Koutsaftis and Aoyama (2006) [42], a shift towards a larger proportion of CuPT might be taking place. It could therefore be hypothesised that changes in speciation outside the organism is a main mechanism behind all the reported synergistic interactions of metal/metal mixtures among the antifoulants (Figure 4C), apart from the mixture of Cu and Ziram [40].

Uptake rates and transport to the target site. One chemical can affect the uptake rate of the other by for example competition at biological ligands or competitive inhibition of transport proteins, as is often observed for interactions on metal uptake ([35,36] and references herein); though not all studies explicitly describe external ion availabilities, making it difficult to determine whether the interactions measured on internal concentration stem from interactions on bioavailability or on uptake.

Interactions on uptake rates have, however, also been measured for combinations of organic contaminants. When Belden and Lydy (2000) investigated the synergistic interactions between the herbicide atrazine and the organophosphate insecticide chlorpyriphos, they found that the addition of atrazine increased chlorpyriphos uptake by 40% [43]. This increase in contaminant uptake was proposed to be caused by an increased oxygen consumption, leading to higher ventilation rates and thereby higher uptake rates of a contaminant as chlorpyriphos, which is predominantly taken up over the gills. Increased ventilation alone could not explain the observed four-fold increase in toxicity. Hence, though it is likely that many contaminants will increase ventilation rates when the organisms start spending energy metabolizing them, thereby increasing uptake of other contaminants taken up over gills, lungs and tracheid's, the quantitative importance of this extra uptake is most likely of little importance for the more severe synergistic cases reported in the literature.

Potential important effects on uptake was also proposed by Kennaugh et al (1993) in a study on the effect of the known synergist Piperonyl Butoxide (PBO) on the cytochrome P450

mediated metabolic rate of the pyrethroid insecticide Permethrin in permethrin resistant and wildtype *Helicoverpa armigera* [44]. The ability of PBO to break the 20-fold resistance could not be explained by differences in P450 monooxygenase mediated permethrin detoxification rates, since they were identical for the resistant and non-resistant genotypes. Hence, it was proposed that PBO instead affected a P450 mediated "penetration resistance" developed by the resistant strain, making the resistant strain take up less pyrethroid. The proposed effect on uptake rates was, however, never confirmed by actual studies of Permethrin uptake. Hence, the P450 mediated "penetration resistance" is still a hypothesis.

Many of the synergists known to enhance uptake belong to the large group of surfactants and other additives added to formulated pesticides with the exact purpose of enhancing the uptake of the active compounds [45]. As this review has excluded all studies with formulated compounds and surfactants, the database does not include examples on surfactant synergies, despite of their frequent use. Though there is a proven effect of the surfactants on uptake of active compounds when hitting their target at high concentrations, it is likely that most lose their "uptake enhancing" potency when diluted in environmental matrices, even though they might still act as dilute pollutants adding to the overall toxicity according to concentration addition. This is supported by a toxicity study on formulated versus technical herbicides on aquatic plants and algae showing no difference in potency for nine out of ten herbicides [46].

The transport rate of one chemical towards its molecular target can be affected by the presence of another chemical, as is for example the proposed mechanisms behind the strong antagonistic responses often seen in plants when a rapidly acting photosynthetic inhibitor is mixed with a slower acting systemic herbicide [47]. No studies have, to my knowledge, shown that one chemical can actively increase the transport of another chemical to their target.Thist is, nonetheless, the proposed mechanism behind many hypotheses regarding nano-particle facilitated increase in chemical toxicity [48], which we will not touch upon here, and therefore cannot be excluded either for chemical/chemical interactions.

Metabolic enzyme activities. Alternations of metabolic activity that are the most well investigated mechanisms behind observed synergistic patterns. A chemical can either increase or decrease the metabolization rate of another chemical. Decreased metabolization will typically lead to a higher toxicity than expected, when the toxic effect is caused by the unchanged parent compound. In contrast, increased metabolization will increase the toxicity of chemicals which are metabolically activated.

Synergistic interactions involving azole fungicides are most likely all examples of cases where the metabolization of the pesticides is inhibited by the azole. Azole fungicides are known inhibitors of a wide range of P450 monooxygenases, which are enzymes responsible for the phase I metabolization of lipophilic compounds [49], together with a range of biosynthesis processes in both plants and animals [50,51]. Hence, the toxicity of lipophilic insecticides such as pyrethroids are often severely enhanced when mixed with azole fungicides [49,52–55].

The synergistic cases involving cholinesterase inhibitors, which made up 76% of all the synergistic pesticide mixtures, most likely all also involve interactions on metabolism. The dominant mechanisms are, however, different depending on which compounds are involved. Basically three mechanisms can be involved:

First, besides the target enzyme acethylcholinesterase (AChE), organophosphate and carbamate insecticides can also inhibit esterases, which are responsible for phase II metabolization of other xenobiotics, including organophosphates and carbamates themselves [49]. Although having the same mode of action and therefore supposedly following concentration addition, mixtures of some organophosphates and carbamates do act synergistically [56–61] (Figure 3).

Second, organophosphates, from the phosphorothioate and phosphorodithioates class of organophosphates, must be metabolically activated to their more active oxon form in order to inhibit the target site AChE [43]. This means that compounds that can induce the production of P450 monooxygenases, will increase the rate of oxon formation and hence increase the toxicity of the organophosphates. This mechanism has been proposed as being the main mechanism responsible for the cases of synergy between triazine herbicides and organophosphates [43,62]. Belden and Lydy (2000) elegantly showed how the amount of polar metabolites of chlorpyriphos increased in *Chironomus tentans* in the presence of atrazine [43], explaining the majority of the observed synergy. Triazine herbicides have also been shown to induce P450 activity in fish [63,64]. The fact that all the cases of pesticide synergy between triazines and organophosphates include organophosphates belonging to the class of phosphorothioate and phosphorodithioates (chlorpyriphos, diazinon, malathion, methidathion, methyl-parathion) [43,62,62,65–68], or being transformed into one [69,29], indicate that triazine induced P450 induction is the main cause of this synergistic interaction. However, not only triazines induce P450 activity. Many xenobiotics, ranging from polyaromatic hydrocarbons, dioxins and ethanol [49,70] to natural substances in honey and metal ions [71,72] are proven P450 inducers. The synergistic interactions between organo(thio-)phosphates and neonicotenoids in the nematode *Caenorhabditis elegans* were also proposed to stem from P450 induction of neonicotenoids [73]. It even seems as if compounds that inhibit P450 activity at high concentrations induce activity at low concentrations or on a longer time-scale. Azole fungicides have, for example, shown to give protective effects against pyrethroid toxicity at low doses in bees [52] and the aquatic invertebrate *Daphnia magna* (pers.obs.), and have been shown to induce synergy together with organophosphates in birds pre-treated with prochloraz [74–76]. In the bird studies increased metabolism of the organophosphates was measured, strongly indicating P450 induction [75,76].

Third and finally, phosphorothioate organophosphates are known to inhibit some types of P450 monooxygenases, thereby not only affecting phase II but also affecting phase I metabolism of xenobiotics [49]. New studies have shown that the inhibitions and activations of different P450 genes are compound specific [77], as are the xenobiotics affinities for the different monooxygenases [49]. Hence, it is likely that the majority of severe synergistic interactions can be explained by interactions on metabolism. Which types of interactions plays the largest role for specific chemical combinations, and at which concentrations and time-scales the interactions are most severe for different species, is, however, still largely unexplored.

Excretion. As the ability of an organism to excrete a compound is mainly related to its ability to transform xenobiotis to an excretable form, excretion is closely related to metabolization. One exception is active excretion of essential metals as Cu and other ions, for which specific transporters or other excretion systems exist, aiding in keeping internal concentrations within a non-toxic range [78,79]. It could be hypothesised that interactions on these excretion processes could lead to synergistic interactions if they were in some way inhibited, though none of the synergistic mixtures included in this study have proven these mechanisms to be important.

Synergistic Interactions where the Mechanisms are Unknown

Apart from the pesticide and metal examples given above, where mechanisms causing synergistic interactions are, if not proven, then at least suggested, the review also revealed synergistic chemical combinations where the mechanisms are unknown. These were mainly the interactions between PSII herbicides with other PSII herbicides, metals or non-azole fungicides in the antifouling mixtures together with the mixtures of metals and organo-metals (Table S3 in File S1) or simply metals and organic pesticides (Table S4 in File S1).

Photosystem II herbicides did not induce synergy in any of the 33 mixtures performed on plants or algae in the pesticide database (Table S1 in File S1). Hence, it was surprising to find that significant synergy was found in nine of the 21 antifoulant mixtures including PSII herbicides when tested on plants or algae. Five of these nine mixtures were with the metals Cd, Cu and Zn, which were not part of any of the PSII mixture in the pesticide database. A proposed synergistic mechanism between metals and PSII inhibitors in autotrophs could be that metals might prevent the repair of not only damaged PSII complexes, which are constantly repaired during photosynthesis [80], but also the damage caused by the reactive oxygen species (ROS) created by the PSII inhibition and the metals themselves, by interacting with enzymes responsible for the repair. The two synergistic PSII/PSII mixtures were between irgarol and diuron, while the remaining two were between irgarol and chorothalonil or TCMTB. The synergies between irgarol and the two general fungicides, chorothalonil and TCMTB, could be similar to the mechanism proposed for the PSII/metal interactions, as both fungicides create ROS [81] and additionally chlorothalonil conjugates with gluthatione [29], an important ROS scavenger. These hypotheses, however, need to be tested.

The mode of action of PSII inhibitors in heterotrophs is largely unknown, as these organisms lack photosystems. The studies on pesticides revealed that triazines, such as irgarol, can induce P450 activity in heterotrophs, thereby enhancing the effect of the organophosphates which needed to be metabolically activated. A study by Suzuki et al (2004) show that also dichlofluanid and chlorothalonil need activation by P450 monooxygenases to reach their full lipid-oxidation potential, with dichlofluanid being far more potent than chlorothalonil [81]. As tolylfluanid is chemically related to dichlofluanid, it might also have to be oxidised by P450 to be fully activated. A study on fish have shown that also the metal ions Cu^{+} and Pb^{+} can induce P450 activity [72]. Five of the seven synergistic mixtures involving PSII inhibitors or metals together with organics are mixtures of the triazine irgarol or Cu and either dichlofluanid or tolylfluanid. In addition, for the twenty synergistic mixtures with more than two antifoulants, these combinations were present in all but three mixtures. It could therefore be hypothesised that the main mechanism behind the synergy between irgarol or Cu (or CuPT), and the fungicides dichlofluanid and tolylfluanid were irgarol and Cu mediated P450 induction leading to faster activation of dichlofluanid and tolylfluanid. But this hypothesis would have to be tested.diuron and TCMTB also induced synergy together with irgarol in two cases each, in the heterotrophic organisms, but information in terms of possible P450 induced activation of these two compounds has not been found.

Metal induced P450 activity could possibly also play a role in the synergies with the phosphorothioate organophosphates malathion, chlorpyriphos and dimethoate [56,82](Sejerøe 2011) (Table S4 in File S1), but as synergies between metal ions an phosphate organophosphates as dichlorvos, the carbamate carbofuran and the azole penconazole [56]Sejerøe 2011) was also found, other mechanisms are most likely also of importance. Lister et al (2011) found an increased uptake and metabolization rate of chlorpyriphos in the presence of Ni, but data were two variable to say anything definite [83]. However, as both P450 monooxygenases and esterases are important enzymes in many biochemical processes, changes in their activity could also affect uptake, excretion or possibly some of the mechanisms used for inactivation of metals in different organisms, though these hypotheses must be subjected to experimental scrutiny.

Is Synergy of any Importance in Nature?

For synergistic interactions to take place in the environment, interacting chemicals have both to co-occur and to be present at levels high enough to induce the synergy. Co-occurrence does happen, as has been shown for both pesticides and antifoulants [11,59,84]. Looking at the cases presented in this review, however, most experiments showing significant synergy use chemical concentrations in the high $\mu g\ L^{-1}$ to $mg\ L^{-1}$ range, which is considerably above the concentrations most often monitored in the environment ($pg\ L^{-1}$ to the low $\mu g\ L^{-1}$ range) [11,59,84]. Very few studies though use realistic concentration ranges, Laetz et al (2009) being such an exception. It is, however, likely that a threshold for synergistic interactions exists for most synergists, and that only a few proven synergists will act as synergists at any endpoint when diluted down to realistic environmental levels. This loss of efficiency as a synergist has for example been shown for piperonyl butoxide (PBO), a known P450 inhibitor, when used to formulate pyrethroid insecticides for mosquito control [85]. In this case, adding the synergist to the aquatic environment did not increase the efficacy of the insecticide towards an aquatic crustacean. Another case, however, showed PBO to enhance pyrethroid toxicity down to concentrations as low as $25\ \mu g\ L^{-1}$ [86]. Hence, more data is needed to determine if a lower threshold for synergists interfering with metabolic processes do exist. In these studies it will be important to include sub-lethal endpoints such as growth and reproduction so that true long term effects on population growth can be estimated.

Conclusion

From the present review of possible mechanisms causing the observed synergies, it can be concluded that interactions on metabolic processes affecting the transformation of xenobiotics seem to be far the most common mechanism of synergy, though interactions on availability and uptake might play an important role for metal/metal synergies. For the synergistic interactions between pesticides, with cholinesterase inhibitors and azole fungicides being present in 95% of the described synergistic cases, the chemical groups causing synergy can be well defined. For the antifoulants the pattern was less clear, primarily due to the lack of knowledge on the interference of the compounds with metabolic processes. However, knowing that most synergistic interactions most likely stem from interactions on metabolic processes, it would be possible to screen for potential synergists using either *in vitro* assays on P450 monooxygenase or esterase inhibition potential, or by investigating metabolization kinetics *in vivo* in representative test species; though the latter is quite labour intensive.

In the introduction it was stated that if we could identify the groups of chemicals that are likely to induce synergistic interactions, special precautions could be taken in the risk assessment of these chemicals. The present review shows that some groups of potential synergists can indeed be identified, while others need more research to be specifically defined as synergists.

That said, considering the generally high chemical concentrations needed to induce synergistic interactions, their importance as synergists within naturally occurring exposure scenarios is most likely of a relatively small importance compared to the additive effect of many co-occuring pollutants. Even if one compound enhances the effect of another compound four-fold, it only takes another three compounds of a similar strength to arrive at the same joint toxicity. And considering the complex pollution patterns monitored [13,33,87], the additive effect of the many co-occurring pollutants might likely project a larger hazard than those of the presence of a few synergist. Hence, in a regulatory perspective addressing the cumulative effect of co-occurring chemicals is the first and most important step in providing a more realistic hazard assessment of chemical cocktails in both man and environment.

Supporting Information

Checklist S1 PRISMA 2009 checklist for systematic reviews and meta-analyses.
(DOC)

File S1 Table S1A. Antagonistic and additive pesticide mixtures. All binary antagonistic and concentration additive pesticide mixtures from Belden et al (2007) sorted with increasing Model Deviation ration (MDR). The synergistic mixtures from Belden et al (2007) are included in Table S1B. For information on species tested, endpoint and original references, please see Belden et al (2007), Supplementary material, Table 1. Table S1B. Synergistic pesticide mixtures. The mixtures are sorted with increasing MDR and including information on the test species, its phylum, subphylum or class, the endpoint tested and the reference of the original study. The synergistic mixtures also included in Belden et al (2007) are given in bold. In the cases where the same mixtures were repeated on the same organism in independent experiments, MDR-values are given for all experiments and are sorted according to the highest MDR value. One full ray-design is defined as one experiment, even though several mixture ratios were tested. Table S2. Metal mitures. Antagonistic and concentration additive mixtures of metal ions from Vijvers et al (2011) and Xu et al (2011) from which MDR-values could be calculated, sorted with the binary mixtures first and then with increasing MDR. Below are the four synergistic mixtures found of which one mixture, given in bold, was obtained from Vijvers et al (2011). The table includes information on the test species, its phylum, subphylum or class, the endpoint tested and the reference of the original study. The last three entries are the three extra synergistic mixtures found by the additional database study. Table S3. Mixtures of antifoulants. All mixtures of antifoulants (Antif) from which MDR-values could be calculated, sorted with the binary mixtures first and then with increasing MDR. The table includes information on the test species, its phylum, sub-phylum or class, the endpoint tested and the reference of the original study. For full chemical names and chemical class and mode of action of the antifaulants, please consult Table 1 in the manuscript. The following names are abbreviated: Irgarol1051 (Irgarol), Seanine211 (Seanine), Chlorothalonil (Chlorot.), Dichlofluanid (Dichlo), Tolylfuanid (Tolyl). Table S4. Additional synergistic mixtures. Synergistic mixtures between metals and organic compounds which did not fit into any of the three categories; pesticides, metals or antifoulants, sorted with increasing MDR. The table includes information on the test species, its phylum, subphylum or class, the endpoint tested and the reference of the original study.
(DOCX)

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Author Contributions

Conceived and designed the experiments: NC. Wrote the paper: NC.

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Letter

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Matrix Complications in the Determination of Radium Levels in Hydraulic Fracturing Flowback Water from Marcellus Shale

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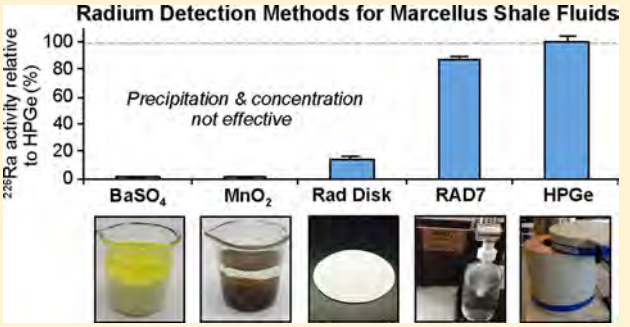
Supporting Information

ABSTRACT: The rapid proliferation of horizontal drilling and hydraulic fracturing for natural gas mining has raised concerns about the potential for adverse environmental impacts. One specific concern is the radioactivity content of associated "flowback" wastewater (FBW), which is enhanced with respect to naturally occurring radium (Ra) isotopes. Thus, development and validation of effective methods for analysis of Ra in FBW are critical to appropriate regulatory and safety decision making. Recent government documents have suggested the use of EPA method 903.0 for isotopic Ra determinations. This method has been used effectively to determine Ra levels in drinking water for decades. However, analysis of FBW by this method is questionable because of the remarkably high ionic strength and dissolved solid content observed, particularly in FBW from the Marcellus Shale region. These observations led us to investigate the utility of several common Ra analysis methods using a representative Marcellus Shale FBW sample. Methods examined included wet chemical approaches, such as EPA method 903.0, manganese dioxide (MnO_2) preconcentration, and 3M Empore RAD radium disks, and direct measurement techniques such as radon (Rn) emanation and high-purity germanium (HPGe) gamma spectroscopy. Nondestructive HPGe and emanation techniques were effective in determining Ra levels, while wet chemical techniques recovered as little as 1% of ^{226}Ra in the FBW sample studied. Our results question the reliability of wet chemical techniques for the determination of Ra content in Marcellus Shale FBW (because of the remarkably high ionic strength) and suggest that nondestructive approaches are most appropriate for these analyses. For FBW samples with a very high Ra content, large dilutions may allow the use of wet chemical techniques, but detection limit objectives must be considered.



INTRODUCTION

New horizontal drilling technologies combined with hydraulic fracturing have the potential to unlock significant reserves of previously unrecoverable shale-bound natural gas around the world.[1−3] However, the rapid proliferation of these drilling techniques has sparked debate over the potential for undesirable environmental impacts. One specific concern is the radioactivity content of produced fluids and "flowback" wastewater (collectively termed FBW), which is typically enriched in naturally occurring radium (Ra) isotopes.[4,5] For example, concentrations of ^{226}Ra and ^{228}Ra in FBW from the Marcellus Shale formation in the United States (underlying New York, Pennsylvania, West Virginia, and Ohio) have been reported in peer-reviewed studies to be as high as 626 and 96 Bq/L, respectively.[4] Although these levels are not sufficient to cause acute radiotoxicity, the large volumes and high ionic strength of FBW can overwhelm wastewater treatment facilities,[6] giving rise to radioactive contamination downstream of wastewater treatment plant discharges. For example, a recent peer-reviewed report documents ^{226}Ra contamination of approximately 200 times background in sediments downstream of a wastewater treatment plant in Pennsylvania.[7] Given the magnitude of Marcellus FBW waste (>5 billion L in 2014 alone),[8] operators and government agencies are considering

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regulations to monitor wastewaters to ensure appropriate radiation and environmental protection strategies are in place.

One challenge to effective radiation and environmental protection for these activities is obtaining an accurate assessment of the radioactivity concentration of Ra isotopes in samples of FBW. Methods for quantitating isotopic Ra radioactivity in FBW have not been validated, and few peer-reviewed data sets on this topic are available. Several studies have referred to data originating from the Pennsylvania Department of Environmental Protection, which for some samples quantitated ^{226}Ra and ^{228}Ra by routine drinking water methods, specifically EPA method 903.0 and EPA method 904.0.[4,5,9] In another example, the New York State Department of Environmental Conservation proposed in 2009 (revised in 2011) that all FBW must be measured for radioactivity (before discharge) using EPA method 903.0 (alpha-emitting Ra isotopes in drinking water) and EPA method 904.0 (^{228}Ra in drinking water) to quantify ^{226}Ra and ^{228}Ra (page 6-61).[10] Although wet chemical methods are robust for drinking water, because of the remarkably high ionic strength of FBW (particularly from the Marcellus Shale), the reliability of methods such as EPA method 903.0 and EPA method 904.0 is questionable for analysis of FBW. Thus, the goal of this study was to investigate the utility of several methods for analysis of Ra isotopes in a representative sample of Marcellus Shale FBW. We explored $BaSO_4$ coprecipitation (EPA method 903.0), manganese dioxide (MnO_2) preconcentration, a rapid 3M Empore RAD radium disk approach, analysis of ^{226}Ra via radon (^{222}Rn) gas emanation using a portable RAD7 electronic Rn spectrometer, and high-purity germanium (HPGe) high-resolution gamma spectroscopy. Our results suggest strongly that nondestructive spectroscopic techniques are most appropriate for analysis of high-ionic strength FBW.

MATERIALS AND METHODS

General. All reagents were ACS grade or higher. The State Hygienic Laboratory at the University of Iowa complies with standards of operation and quality assurance required for accreditation by the U.S. National Environmental Laboratory Accreditation Program (NELAP). All radioactivity values are decay-corrected to 2:18 p.m. (CST) on May 15, 2013. Unless otherwise stated, all uncertainties are "standard uncertainties", corresponding to a one-uncertainty interval based on the standard deviation of multiple measurements or an estimate thereof, according to principles adhered to by international standards bodies.[11]

Flowback Wastewater Sample. The University of Iowa State Hygienic Laboratory (SHL) received a 200 L drum of Marcellus Shale FBW from northeastern Pennsylvania. The sample was extracted from a 2100 m deep, horizontally drilled well, which was hydraulically fractured with approximately 35000 m^3 of hydraulic fracturing fluid in early 2012. The sample was received May 7, 2013. Prior to radium quantitation, analysts at SHL determined the chemical composition by standard environmental techniques (Table S1 of the Supporting Information).

Surrogate Matrix. A surrogate blank matrix was prepared for quality assurance/quality control (QA/QC) analysis using reagent grade NaCl, KCl, $MgCl_2{\cdot}6H_2O$, $CaCl_2$, $SrCl_2{\cdot}6H_2O$, $BaCl_2{\cdot}2H_2O$, and $FeCl_3$ in deionized water (dH_2O). The surrogate was prepared to match as closely as possible the FBW sample matrix based on mass spectrometry analysis (Table S1 of the Supporting Information).

Methods of Analysis Tested. *$BaSO_4$ Coprecipitation.* We attempted to use the EPA method 903.0 isotopic Ra in drinking water method of analysis. However, the addition of 20 mL of 18 M H_2SO_4, prescribed by this method, formed excessive volumes of precipitate, which rendered the approach intractable. In a further attempt to utilize the technique, we developed a modified EPA method 903.0 protocol. Briefly, three 100 mL samples of FBW, three 100 mL surrogate samples spiked with 3.7 Bq of ^{226}Ra, and one 100 mL surrogate blank were diluted to 1 L with dH_2O. EPA method 903.0 was then followed with two modifications: (1) only 0.5 mL of 1 M H_2SO_4 was added at the precipitation step (rather than the prescribed 20 mL of 18 M H_2SO_4), and (2) the Ba carrier was omitted. Counting sources were prepared according to the EPA method 903.0 protocol and were counted on a gas flow proportional counter (Berthod LB 770) for 50 min as prescribed by the method.

3M Empore RAD Radium Disks ("RAD disks"). RAD disks (3M, Eagan, MN) are wide-area (47 mm diameter) filter-based materials impregnated with a chromatographic extractant that is designed to selectively remove Ra from aqueous samples.[12] In our attempt to employ this technology for FBW analysis, three 50 mL samples of FBW were diluted to 500 mL with dH_2O and filtered through RAD disks, according to the manufacturer's recommendations. Disks were counted for 17 h on a Canberra HPGe detection system, calibrated to a 47 mm diameter wide-area filter geometry [Eckert and Ziegler (E&Z) 93471]. Filtrates were collected in 0.5 L Marinelli beakers and counted for 17 h using a 0.5 L liquid geometry, calibrated for energy and efficiency with an identical geometry NIST traceable standard (E&Z 93472). The radioactivity concentrations of ^{226}Ra in the filtrates and filters were determined by the 186 keV peak as described previously.[13,14]

Rn Emanation Measurement by RAD7. Several methods are used frequently to determine ^{226}Ra levels in liquid matrices based on emanation and measurement of ^{222}Rn, including mineral oil extraction and liquid scintillation counting and Lucas cell emanation-based gross-counting techniques. We evaluated the emanation approach using RAD7 (DURRIDGE Co., Inc., Billerica, MA), an electronic radon detector that quantifies isotopic radon activity based on measurement of short-lived alpha-emitting Rn daughters by high-resolution alpha spectrometry.[15] All materials for the RAD7 experiments were purchased from DURRIDGE and used according to the manufacturer's instructions or in consultation with the manufacturer. Briefly, glass vials (250 mL) were filled with FBW and hermetically sealed for at least 30 days to reach secular equilibrium between ^{226}Ra and ^{222}Rn. Samples were analyzed using the RAD H_2O accessory package, with the following minor manufacturer-recommended modification: an empty desiccant tube was inserted into the apparatus to control foaming of FBW and the relative humidity in the detector. Activities were calculated by the preprogrammed WAT250 protocol, adjusted for relative humidity with DURRIDGE Capture version 5.2.2, and decay corrected. QA/QC checks with samples of known ^{226}Ra activity (4, 40, and 100 Bq/L), analyzed as described above, were in agreement with the manufacturer's calibration of the detector.

MnO_2 Preconcentration. The method of preconcentration of Ra on MnO_2 has been used often for effective Ra isotopic analysis of water samples.[16−18] For our evaluation, 30 mg of $KMnO_4$ was added to 250 mL of acidified FBW and the pH was adjusted to 7−8 with 6 M ammonium hydroxide (NH_4OH) to form MnO_2. Precipitates were filtered on 0.45

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μm cellulose nitrate filters (Whatman). The filtrate was transferred to 250 mL glass vials, diluted with dH_2O, and sealed for at least 30 days. Precipitates and filters were digested in concentrated HNO_3, transferred to 250 mL glass vials, neutralized with 6 M NH_4OH, and sealed for at least 30 days. Activities of ^{222}Rn were then determined by RAD7 by following the manufacturer-recommended protocol.[15] In this way, the efficiency of the MnO_2 in sequestering Ra could be assessed by the difference between the filtrate concentration and the filtered MnO_2 levels.

Gamma Spectroscopy. HPGe gamma spectrometry analysis of FBW was conducted according to routine procedures using NIST traceable standards. Briefly, HPGe gamma spectrometers were calibrated to (1) a 3 L Marinelli beaker liquid geometry (E&Z 93474), (2) a 47 mm wide-area filter geometry (E&Z CRM 93471), or (3) a 0.5 L Marinelli beaker liquid geometry (E&Z 93472), as appropriate. QA/QC included linearity and efficiency checks performed three times per week and weekly background counts. Once the bulk sample of FBW had been received, 3 L was transferred to a 3 L Marinelli beaker. Because of the settling of ultrafine particulate matter, 51 g of Bacto Agar (BD 214010) was added. The sample was heated to a low boil and then slowly cooled to form a homogeneous suspension. Gamma emissions were measured for 17 h on a 30% efficient ORTEC (Ametek, Oak Ridge, TN) HPGe, calibrated to a 3 L liquid Marinelli geometry (E&Z 93474). After 62 days, the sample was recounted on an 18% Canberra HPGe gamma detector (calibrated to E&Z 93474) to confirm ingrowth of short-lived daughters, ^{214}Pb, ^{212}Pb, and ^{214}Bi. Spectral analysis was performed using ORTEC GammaVision version 6.08 with a library that included NORM expected in FBW. Emission energies, half-lives, and their uncertainties were extracted from the National Nuclear Data Center (http://www.nndc.bnl.gov/nudat2/).[19]

RESULTS AND DISCUSSION

Chemical Matrix. Analysis of the elemental composition revealed the FBW used for this study has high concentrations of monovalent and divalent ions, solids, and transition metals (Table S1 of the Supporting Information). Briefly, concentrations of monovalent and divalent ions were as follows: 147000 mg/L Cl, 36000 mg/L Sr, 29000 mg/L Na, 13000 mg/L Ca, 9000 mg/L Ba, 850 mg/L Mg, and 160 mg/L K. The concentration of the total dissolved solids was 278000 mg/L and that of the total suspended solids 780 mg/L. The concentrations of Pb, Fe, and Mn were 1.0, 43, and 3.4 mg/L, respectively. The high concentrations of solids, Sr, and Ba are characteristic of Marcellus Shale FBW reported previously.[5,9,20]

Barium Sulfate Coprecipitation. The first method we investigated is commonly used for Ra concentration determinations in drinking water, i.e., EPA method 903.0. This method involves the addition of $BaCl_2$ and H_2SO_4 to precipitate Ra as $Ba(Ra)SO_4$. We found that following the procedure as written results in copious, unmanageable quantities of precipitate. Because of excessive precipitate formation, we were unable to use EPA method 903.0 to quantify Ra activities in samples as small as 10 mL. To determine whether a modified form of EPA method 903.0 would be useful, we reduced the quantity of H_2SO_4 by a factor of 720 and diluted the salt concentration by a factor of 10. This reduced the final precipitate to acceptable mass ranges but resulted in poor recovery of Ra. Activities of ^{226}Ra surrogate spikes were calculated to be <1% of spiked activity. Similarly, activities of FBW were calculated to be <1% of the ^{226}Ra activity determined by HPGe. We interpret this finding as illustrating that the similar chemistry of Ra and Ba prevents the use of $Ba(Ra)SO_4$ precipitation in samples with large Ba:Ra mass ratios (nearly $1:10^9$ in this sample), as are commonly found in FBW. Thus, our data suggest that $Ba(Ra)SO_4$ coprecipitations are not appropriate for analysis of FBW in general and (in particular) for the analysis of Marcellus Shale FBW. These laboratory findings may also explain observed difficulties experienced by wastewater treatment facilities (using similar coprecipitation approaches) in removing Ra from FBW,[7] potentially leading to improved wastewater treatment strategies.

3M Empore Radium RAD Disks. RAD disks have been used successfully to concentrate Ra from aqueous environmental samples.[21,22] The use of the RAD disk technology is appealing, because the approach is rapid, with fewer wet chemical steps than $BaSO_4$ coprecipitations.[21] The manufacturer reports the disks recover >95% of Ra in samples with high concentrations of divalent cations, although a published peer-reviewed upper limit of metal concentration has not been established, to the best of our knowledge.[12] When we tested 50 mL of FBW, diluted 10-fold in dH_2O, the recovery of ^{226}Ra was 13 ± 1% (n = 3) of values obtained by direct measurement using HPGe. Although recovery was low, others have suggested radioactive tracers, such as ^{133}Ba or ^{225}Ra, could be used for isotope dilution-based approaches.[22,23] Nonetheless, the efficiency of the RAD disk appears to be questionable for high-ionic strength FBW, and a more thorough study is needed to establish an upper limit of ionic strength within which the technology can be reliably employed for analysis of FBW.

MnO_2 Preconcentration. Manganese dioxide is used often to preconcentrate Ra for radiochemical analysis.[16−18] However, we hypothesized the divalent-rich matrix of FBW would hinder the efficiency of the approach. To test this assertion, we performed MnO_2 preconcentration of FBW to determine if ^{226}Ra would sorb to MnO_2 or remain in solution. Results indicated that MnO_2 scavenged <1% of ^{226}Ra from the FBW (i.e., the filtrate contained >99% of the ^{226}Ra). Although preconcentration with MnO_2 is useful for certain complex matrices, high-ionic strength brine, such as that from the Dead Sea, has been reported to reduce Ra recovery on MnO_2-impregnated acrylic fibers.[24] Similarly, our results indicate that the high concentrations of divalent cations in FBW interfere with the use of MnO_2-based preconcentration for the analysis of FBW.

Rn Emanation Measurement by RAD7. RAD7 is a sturdy, portable, electronic radon detector that can be used to measure ^{222}Rn and ^{220}Rn (decay products of ^{226}Ra and ^{224}Ra, respectively) in environmental water samples in field and laboratory environments.[25−27] The system can be used to measure unsupported ^{222}Rn and ^{220}Rn levels in water, by immediate measurement, as well as ^{226}Ra and ^{224}Ra by hermetically sealing water samples and allowing sufficient time for Rn radioactivity products to reach radioactive equilibrium. When the RAD H_2O closed-loop system is used, the RAD7 can measure ^{222}Rn activities in water from <0.37 to 14800 Bq/L.[15] Measurement of ^{226}Ra in FBW via ^{222}Rn emanation is advantageous relative to wet chemical analysis techniques because Rn gas can be stripped from complex chemical matrices, allowing for sample volumes larger than and detection limits lower than those of precipitation methods. On the other hand, for analysis of the Marcellus Shale FBW sample

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described here, controlling foam produced during the Rn gas-stripping process using RAD7 was a challenge. To alleviate the problem, we inserted an empty desiccant tube between the sample vial and the filled desiccant tube. Another (related) challenge was controlling the humidity in the detector chamber, which can reduce the counting efficiency of the RAD7 device. When adjusted for humidity using the DURRIDGE Capture software and for volume introduced by the empty desiccant tube, the radioactivity level of the ^{226}Ra level in FBW observed in this study was 610 ± 10 Bq/L (n = 3). This estimation of ^{226}Ra may differ from HPGe values for several reasons, including the effects of brine on the solubility of Rn.[28] Additionally, modifications to the RAD7 may be necessary to reduce possible interference from dissolved gases.[29] A more rigorous examination of these parameters is ongoing in our laboratory. If analysis of large numbers of samples is required rapidly, in a high-throughput laboratory environment, mineral oil-based ^{222}Rn extraction/emanation and liquid scintillation counting and Lucas cell-based emanation techniques can be employed to improve throughput. A potential drawback of ^{226}Ra measurements by this method is the holding time for radon ingrowth. The holding time may be as short as 4 days if the sample is purged prior to being hermetically sealed; however, because sample foaming prevented complete purging, we chose to hold for 30 days to establish secular equilibrium. Thus, for samples with sufficient ^{226}Ra radioactivity content, direct measurement by HPGe gamma spectroscopy (as described below) may offer a simpler solution to achieving statistical significance in radioactivity quantitation.

HPGe Gamma Spectroscopy. HPGe gamma spectroscopy is well-established for the determination of the levels of ^{228}Ra, ^{226}Ra, and ^{224}Ra in environmental samples, with achievable detection limits depending primarily on sample size, detector efficiency, and available counting time.[30−32] Within these constraints, given that the Ra isotopic concentration of Marcellus Shale FBW is relatively high, the clear advantage of HPGe gamma spectroscopy for the analyses here is the simplicity of sample handling (i.e., no wet chemistry required; apart from the addition of agar and moderate heating, no alterations were made to the sample). Thus, high-ionic strength FBW samples can be measured directly, and samples can be stored for future analysis (if required). Radium activities observed in the representative FBW sample used for this assessment are well in excess of typical environmental levels in natural surface waters reported in this region of Pennsylvania [^{226}Ra, 670 ± 3 Bq/L; ^{228}Ra, 76 ± 1 Bq/L (Table 1)].[7] ^{228}Ra activities were determined by integration of ^{228}Ac radioactive product peaks (911 and 338 keV), with an achievable minimal detectable activity (MDA) of 0.6 Bq/L under the counting conditions employed. The ^{226}Ra value was determined on the basis of a direct measurement of the 186 keV ^{226}Ra peak, with an achievable MDA of 3 Bq/L. Although interference from ^{235}U gamma ray emission in the 186 keV region is possible,[33] preliminary analysis of natural U isotopes ^{238}U, ^{235}U, and ^{234}U by alpha spectrometry reveals activities of <0.01 Bq/L of FBW. Thus, the contribution of ^{235}U to the 186 keV region is negligible for these analyses. Very little natural U is extracted during the hydraulic fracturing process because of the insolubility of U under the reducing conditions at depth in the shale deposit. Further studies are required to develop a detailed understanding of the behavior of U in unconventional, drilling-derived solid waste and in the FBW use cycle (a topic of current research in our laboratories). Lower radioactivity concentrations of ^{228}Ra (and decay products ^{224}Ra, ^{212}Pb, and ^{208}Tl) relative to those of ^{226}Ra (and decay products ^{214}Pb and ^{214}Bi) can be explained by a lower concentration of natural Th (^{232}Th relative to natural ^{238}U) at depth in the shale deposit. Importantly, regardless of the decay product equilibrium and/or disequilibrium associated with FBW, direct measurement of ^{226}Ra requires no holding time and can be measured directly by HPGe via the 186 keV gamma ray emission of ^{226}Ra. When possible, measurements conducted using the 186 keV peak can be confirmed by measuring ^{226}Ra decay product ingrowth after the proper holding time. The radioactive equilibrium of ^{228}Ac ($t_{1/2}$ = 6 h) with ^{228}Ra is reached in ∼36 h for these analyses. While ionic strength differences between control standards and high-ionic strength samples under analysis can contribute to inaccuracies in Ra isotopic measurements due to density differences, our analysis of surrogate FBW indicates no significant contribution.[34] Differences in the ^{226}Ra radioactivity level determined by the RAD7 emanation method may be the result of inaccuracies in humidity corrections applied, and an improved apparatus can easily be envisioned for efficient field studies by this emanation technique (a topic of ongoing research in our laboratories). Nonetheless, our results strongly suggest that wet chemical techniques (e.g., EPA method 903.0) are unlikely to be reliable for the analysis of high-ionic strength FBW, and direct measurement by emanation techniques and HPGe spectroscopy is recommended for accurate assessments. For FBW samples with a very high Ra content, large dilutions may be applied (to dilute the ionic strength) to allow the use of wet chemical techniques, but detection limit data quality objectives must be considered.

Table 1. Comparison of ^{226}Ra Quantitation Methods

method	sample description	volume (L)	^{226}Ra recovery (%)	^{226}Ra (Bq/L)
$BaSO_4$ coprecipitation	surrogate spike (3.7 Bq of ^{226}Ra)	0.1	<1[b]	0.15 ± 0.03[d]
	FBW[a] and 0.5 mL of 1 M H_2SO_4	0.1	<1[b,c]	1.9 ± 0.4[d]
Empore RAD disk	FBW diluted 10-fold, RAD disk	0.05	13 ± 1	96 ± 8[d]
	FBW diluted 10-fold, supernatant	0.05	87 ± 1	642 ± 1[d]
MnO_2 concentration	FBW and 10 mg of Mn, precipitate	0.25	<1	0.9 ± 0.3[d]
	FBW and 10 mg of Mn, supernatant	0.25	>99	600 ± 20[d]
RAD7	FBW	0.25	91[c]	610 ± 10[d]
HPGe	FBW and Bacto Agar	3	100	670 ± 26[e]

[a]FBW, flowback water. [b]Assuming a 100% efficiency of Ba recovery. [c]Relative to the HPGe 186.2 keV peak. [d]Uncertainties are reported as the standard deviation of three counts. [e]Counting uncertainty.

■ ASSOCIATED CONTENT

Supporting Information

Chemical composition of flowback water (Table S1). This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

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OCS Study
MMS 2002-050

Effects of Produced Water on Complex Behavior Traits of Invertebrate Larvae

Final Technical Summary

Final Study Report

U.S. Department of the Interior
Minerals Management Service
Pacific OCS Region

Effects of Produced Water on Complex Behavior Traits of Invertebrate Larvae

Final Technical Summary

Final Study Report

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FINAL TECHNICAL SUMMARY

STUDY TITLE: Effects of Produced Water on Complex Behavior Traits of Invertebrate Larvae

REPORT TITLE: Effects of Produced Water on Complex Behavior Traits of Invertebrate Larvae

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KEY WORDS: ecotoxicology, produced water, oil drilling, invertebrate larvae, bryozoan, *Watersipora* spp, *Bugula neritina*, *Haliotis rufescens*, *Schizoporella unicornis*, sub-lethal impact, pulsed exposure, carry-over effects.

BACKGROUND: Produced Water (PW) is a by-product of oil drilling and in the Southern California region a common form of disposal of PW is by direct discharge into the ocean from a platform-based diffuser. Many compounds and elements in PW are known to have toxic effects

on a number of organisms however these toxic effects are not always lethal, even in static conditions.

Short, spiked exposure sees larvae exposed to a pulse of PW for a short period of time and is unlikely to produce lethal effects, however it may result in a series of important sub-lethal effects for organisms in various stages of their lifecycles. It is now generally believed that the discharge of produced water can cause severe, generally sub-lethal, effects to organisms over distances well beyond that predicted by plume dilution models. Plume measurement and modeling has shown that it is more than possible for larvae in the water column 1km away from a diffusion source to contact PW at 1% of it's original concentration.

The early life history stages of invertebrate larvae are very important developmental phases. Some invertebrates and algae have been shown to be particularly susceptible to any negative effects of contaminants during these developmental phases. The impacts seen on larval behavior have included swimming behavior, cue-recognition, settlement and larval survival. Little work has been published that follows the sub-lethal impacts on larvae through to their adult phase. The ecological consequence of carrying-over impacts to the adult phase from sub-lethal impacts on larvae have been rarely tested.

OBJECTIVES: One of the major aims of this project was to test the assumption that sub-lethal impacts on larvae can, and do, carry-over into the adult phase of the invertebrate life-cycle. By exposing invertebrate larvae at an early developmental stage and following their development through to adulthood and beyond, it is possible track the impact of early exposure to PW.

Other aims were more broad: to expand the number of larvae exposed to, and types of sub-lethal impacts assessed from exposure to PW.

DESCRIPTION: By exposing different types of larvae to PW at various stages of their development, we had a number of outcomes:

- First, we expanded the number and type of larvae tested for sub-impacts from pulsed exposure to PW.

- Second, we tested the types of sub-lethal impacts that can occur on invertebrate larvae from exposure to PW. By doing so, we tested for the impact of PW on a number of sub-lethal endpoints, including swimming behavior, cue-recognition, attachment and metamorphosis.

- Finally, we followed some species of larvae through their development into adults and tested for any carry-over impacts for a range of endpoints, including growth, competitive ability, reproduction and survival.

The project tested a wide range of invertebrate larvae, including the bryozoans *Watersipora* spp, *Bugula neritina, Schizoporella unicornis*, the red abalone *Haliotis rufescens*, the sea star *Asterina miniata* and the ascidian *Botrylloides* spp. for carry-over effects from the sub-lethal impact of exposure to produced water as larvae. This broad range represents four different phyla of both

introduced and local species as well as a broad range of types of larval development. All exposures were short-term, spiked exposure in still water conditions in the lab.

SIGNIFICANT CONCLUSIONS:

- All exposures were short-term, spiked exposure in still water conditions in the lab.
- Not all experiments on all invertebrate larvae were successful.
- Those experiments that were successful showed, in broad terms, that there was little evidence for strong sub-lethal effects on the growth, competitive ability or reproductive output of those invertebrates successfully studied. However sub-lethal impacts did occur.
- Where mortality occurred, it tended to be larger in colonies of bryozoans in treatments exposed to concentrations of 10% PW.
- Many and varied sub-lethal impacts were found from exposing larvae to a range of PW concentrations (with 10 % PW having the greatest and most consistent impact).
- Sub-lethal impacts include decreases in swimming capacity, slowed metamorphosis, altered attachment and settlement behaviors, and delayed opercula development.
- Not all invertebrate larvae showed all impacts, nor were tested for the full range of endpoints.
- There was little evidence for strong carry-over effects to adulthood of larval PW exposure, with some caveats.

STUDY RESULTS: The results of this study are fully contained within the Report "Effects of Produced Water on Complex Behavioral Traits of Invertebrate Larvae" presented to MMS.

STUDY PRODUCTS: A number of presentations (6) were given during this work as both invited and conference seminars. Three posters were also given at a range of conferences. The details of all these were contained in the annual reports written for MMS.

There are three manuscripts in preparation from work down during this project:

> Boxshall, AJ and PT Raimondi, The sub-lethal impact on *Watersipora subtorquata* adults from exposing larvae to a toxicant. *In prep. A*
>
> Boxshall, AJ, and PT Raimondi, Carry-over effects on adults from exposing *Bugula neritina* and *Schizoporella unicornis* larvae to sub-lethal toxicant. *In prep. B*
>
> Boxshall, AJ and PT Raimondi, Impacts on adult *Phragmatapoma californica* of exposing larvae to a sub-lethal toxicant. *In prep. C*

FINAL STUDY REPORT

Introduction

Produced Water (PW) is a by-product of oil drilling and in the Southern California region a common form of disposal of PW is by direct discharge into the ocean from a platform-based diffuser. The actual content of PW is very variable on both the spatial (regionally, locally) and temporal (daily from the same field) scales. The salinity of many PWs in California is generally around the mid- to high-20's ppt. PWs can contain a large number of different compounds and elements, including polycylic aromatic hydrocarbons (PAH - e.g., benzene, toluene, naphthalene, phenols), metals (e.g., As, Cr, Ni, Ag, Cd, Cu, Pb, Se, Ba), and other compounds (e.g., cyanides and ammonia). Many of these compounds and elements are known to have toxic effects on a number of organisms however these toxic effects are not always lethal, even in static conditions (e.g., Ray and Engelhardt 1993).

If larvae are entrained in a low concentration plume of PW, exposure could be on-going. However, plume dilution models suggest that many invertebrate larvae are likely to undergo spiked, rather than on-going, exposure to PW (e.g., Washburn *et al* 1999). A short, spiked exposure sees larvae exposed to a pulse of PW for a short period of time. This form of exposure is unlikely to produce lethal effects, however it may result in a series of important sub-lethal effects for organisms in various stages of their lifecycles (Raimondi and Schmitt 1993, Reed and Lewis 1994).

Field studies in the early 1990s in the southern California Bight challenged the belief that the discharge of produced water from oil drilling platforms had little or no effect on organisms in the water column (e.g., Raimondi and Schmitt 1993, Krause *et al* 1993, Reed and Lewis 1994, Reed *et al*. 1994). This belief was based, in part, on the idea that the harmful components of PW have relatively short residence times in the water column. Based on acute (lethal) laboratory tests, the water-soluble contaminants in PW are believed to be diluted rapidly to levels well below those suspected to cause meaningful biological responses. However, the results of the studies noted above support the idea that the discharge of produced water can cause severe, generally sub-lethal, effects to organisms over distances well beyond that predicted by plume dilution models.

Washburn *et al* (1999) modeled and measured the movement of diffused PW in the Santa Barbara channel. They found that the minimum initial dilution from the diffuser was approximately 100 times in summer in a zone within approximately 80m of the diffuser source. The dilution rate increased in winter to approximately 500 times. However, from modeling work they also found it was possible to get patches of PW up to 1000m from a diffusion source with time-averaged dilution factors of 100 and 1000 for summer and winter (respectively). Hence, it is more than possible that larvae in the water column even 1km away from a diffusion source could contact PW at 1% of it’s original concentration.

The early life history stages of invertebrate larvae are very important developmental phases. Some invertebrates and algae have been shown to be particularly susceptible to any negative effects of contaminants during these developmental phases (Capuzzo 1987, Raimondi and Reed

1995). Bryozoan (Raimondi *et al* 1997) echinoid (Krause *et al*. 1993) and molluscan larvae (Raimondi and Schmitt 1993), and even algal spores (Reed and Lewis 1994) that are contaminated during this developmental phase show behavioral effects later in the larval phase.

The impacts seen on larval behavior have included swimming behavior, cue-recognition, settlement and larval survival. Little work has been published that follows the sub-lethal impacts on larvae through to their adult phase. There is a suggestion that the metamorphosis that occurs between the invertebrate larval and adult phases can be thought of as "a new beginning" (e.g., Pechenik 1999, Pechenik *et al*, 2001). Clearly, if this is the case, sub-lethal impacts thought to be important for larvae may not be as important from the perspective of the adult organisms. Which the carry-over of impacts to the adult phase, the ecological consequence of sub-lethal impacts on larvae may be diminished. This question has been rarely tested.
One of the major aims of this project has been to test the assumption that sub-lethal impacts on larvae can, and do, carry-over into the adult phase of the invertebrate life-cycle. By exposing invertebrate larvae at an early developmental stage and following their development through to adulthood and beyond, it is possible track the impact of early exposure to PW.

By exposing different types of larvae to PW at various stages of their development, we tested a number of outcomes.

- First, we expanded the number and type of larvae tested for sub-impacts from pulsed exposure to PW.

- Second, we tested the types of sub-lethal impacts that can occur on invertebrate larvae from exposure to PW. By doing so, we tested for the impact of PW on a number of sub-lethal endpoints, including swimming behavior, cue-recognition, attachment and metamorphosis.

- Finally, we followed some species of larvae through their development into adults and tested for any carry-over impacts for a range of endpoints, including growth, competitive ability, reproduction and survival.

By following invertebrates from release through metamorphosis to adulthood as a competing member of a marine assemblage, we can measure the impact of early PW exposure on a different component of the invertebrate's growth or interactions. Despite surviving the initial exposure to PW, the ecological impact of PW exposure on both the organism itself and surrounding organisms is relatively unknown (e.g., see Schüürmann and Market 1997).

In this project, we tried to use a wide range of invertebrate larvae, including the bryozoans *Watersipora* spp, *Bugula neritina, Schizoporella unicornis*, the red abalone *Haliotis rufescens*, the sea star *Asterina miniata* and the ascidian *Botrylloides* spp (see Methods for details on each). This broad range represents four different phyla of both introduced and local species as well as a broad range of types of larval development. Not all tests listed above were done with each invertebrate.

Methods

General Comments

In general, we did similar experiments on various invertebrate larvae. Details of the invertebrates are below (see "Organisms") where a broad overview of the culturing techniques and origins of the various animals is presented.

In all cases, all larval exposure to PW was short between 45 and 90 minutes and occurred in the laboratory at the Long Marine Laboratory (LML) at the University of California, Santa Cruz. Larval culturing facilities were available at LML, which included constant temperature, cultured algal food and flow through water systems. When larvae culture was required, two methods were used. A sunken, constant-flow method (similar to that for molluscs discussed in Strathmann, 1987) was used as well as raising small batches (>1 larva/ml) of larvae in ≥1l beakers of 0.2 μl filtered seawater. Batches were cleaned and fed cultured algae of various species every other day.

In the following section, details of each experimental are discussed. At many times the same method was used for different larvae. This has been noted where it occurred.

Produced Water

The produced water (PW) used in this project was supplied by the Minerals Management Service (MMS) in California. We did not analyze the exact composition of the PW we used in these experiments. Under our agreement with the MMS, we do not know the exact origin of the platform/s from which the PW was taken. We do know there were two samples collected from different platforms on different days in southern California, most likely from within or near the Santa Barbara channel. There was one collection in 1997 and another in 1999. Most experiments used the 1997 collection. After collection of the PW, it was stored on ice. In the laboratory, it was frozen in small aliquots and stored at –80°C within 24 hours of collection. The PW used is not representative of all PW, or even all southern Californian PW (see papers in Ray and Engelhardt, 1993 for discussions of the variation in PW composition). The lack of variation in the samples of PW is a potential a source of experimental error.

In all cases, we exposed the larvae to various concentrations of PW ranging from 0% to 10% (and 25% in some pilot tests) of pre-diffuser levels. Note that all PW concentrations are expressed in % of the pre-release concentrations of PW (i.e., as obtained from the platform downstream of the WEMCO) prior to release via a diffuser. PW was diluted in all experiments using 0.2 μm filtered seawater (0.2SW). The 10% PW treatment was included as a positive control as pilot studies indicated we could expect a sub-lethal larval response to this treatment. We did not expect this concentration to be lethal, however we believed it would elicit negative behavioral responses, such as altering swimming and settlement behaviors. For the same reason we included the 25% PW treatment in some pilot studies. A 10% treatment is quite a high concentration of PW and would generally only be found in close proximity to the diffuser array

of an oil platform (< ~10 m; *pers. comm*. Bill Ford, Chevron, 1997). Washburn *et al* (1999) modeled the movement of diffused PW in the Santa Barbara channel near where we understand the test PW was collected. They showed that the minimum initial dilution was about 100 times (i.e., 1% concentration of the raw PW) in summer (about 500 times in winter) in a zone within approximately 80m of the diffuser source. Hence, the 10% treatment should only be present quite close to the diffuser. A 25% concentration would be unlikely more than 1m from the diffuser array and was only used in pilot studies to produce a known larval response. Larvae are extremely unlikely to encounter PW at 25% in the water column.

Organisms

The bryozoan *Watersipora subtorquata*, is now a common introduced fouling organism in sheltered subtidal waters along the California coast (e.g., Rees 2000). Larvae of *W. subtorquata* were used for a large proportion of this project. The bryozoan *Schizoporella unicornis* is a common native species found in sheltered subtidal areas along the Californian coast (Ricketts *et al* 1985). Both bryozoans brood larvae, which are released after exposure to light. Both have an encrusting, clonal growth form, making them a good target species to follow through settlement and subsequent growth as an adult.

Larvae of *W. subtorquata* and *S. unicornis* were individually collected and stored in a communal beaker until exposed to PW within the first 2 hours of release. For all experiments using these bryozoans, the larvae came from multiple unrelated colonies collected from at least 3 different sites within the Santa Cruz Harbor, Santa Cruz, California, USA. At least 40 different adult colony fragments were used per experiment for both bryozoans. The adult *W. subtorquata* used for spawning in different experiments were from very different stocks and from three different seasons, thus reducing the chance we were re-testing the progeny of the same adults three times. *S. unicornis* were collected for a single experiment.

The upright bryozoan *Bugula neritina* is ubiquitous across the globe (Keough 1989) and found commonly in bays, harbors and sloughs in California. Larvae *of B. neritina* are brooded and released from adults after exposure to bright light. Larvae were individually collected and stored in a communal beaker until exposed to PW within the first 2 hours of release. Adults used in these experiments were collected from Elkhorn Slough, Monterey Bay for use in both 1998 and 1999.

The colonial ascidian *Botrylloides* spp broods larvae which can be released after exposure to light. The adults are encrusting and common in sheltered subtidal areas of the California coast (Ricketts *et al* 1985). A similar protocol to that used for collecting the *W. subtorquata* and *S. unicornis* adults was used with *Botrylloides* spp. The adults used came from the Santa Cruz Harbor. We attempted experiments with the *Botrylloides* spp larvae twice in the summer of 1998 and once in 1999 with limited success.

The sea star *Asterina miniata* is common along the Californian coastline in rocky and sandy areas from the intertidal to >250 m (Ricketts *et al* 1985). We did a series of pilot experiments with the sea star in late 1997. These larvae were cultured using the standard 1-methyladenine method (see

Strathmann 1987 for details). Multiple adults used in these experiments were collected from the intertidal around Santa Cruz and raised in aquaria at LML, UCSC.

The red abalone *Haliotis rufescens* is found subtidally in California. Culturing techniques are well-established (see Boxshall 2000 for information). *H. rufescens* larvae do not feed and are in the plankton for 7 days before they are competent to settle. They cue to a peptide associated with the phycobillins in coralline red algal species, which is a mimetic of the neurotransmitter, GABA (Morse and Morse, 1984).

Specific Methods

Watersipora subtorquata

We ran three experiments with similar methods (see Boxshall and Raimondi, *in prep. A*). The methods used for *Watersipora subtorquata* are a template for the methods used for other larvae. Differences will be noted when they occur.

Larvae were exposed for between 50 and 65 minutes to PW of four concentrations (0%, 0.1%, 1% and 10%). We grew the settlers in the lab for between 8 and 12 days and transferred them to the field for monitoring for between 40 and 150 days. For the first two experiments we pooled all larvae within a treatment into one beaker for the short duration of exposure. This is not the most ideal situation and was forced onto us by low numbers of larvae. It can be argued this results in pseudo-replication, particularly as larval behavior can be quite variable (see any paper in McEdwards 1995). It can also be argued that this practice reduces variation in application of a potentially variable toxicant. Except for this 50 - 65 minute period, the larvae and subsequent adults were raised and monitored individually for the duration of the experiments.

To test if this short-term pooling during exposure in the first two experiments resulted in the loss of important information on the variability of larval reactions to PW we artificially formed batches of larvae for the 3rd Experiment and in some experiments with other invertebrates. If exposing larvae in batches resulted in treatment effects that were different between in batches (i.e., a treatment x batch interaction), we need to be careful with interpretations of the first two experiments. There were very few batch x treatment effects across a range of endpoints (see Results for details).

During Exposure

All larvae were exposed in 100ml of 0.2µm filtered seawater (0.2 SW) and PW (at the required dilution) in 250ml plastic beakers (Markson Lab Supplies) in static water conditions in a water bath at 15 -18°C. All beakers were swirled every 10-15 minutes to discourage settlement during exposure. For the three main experiments, PW concentrations ranged between 0% (the Control) and 10% of the raw PW concentration (see Table 1 for details). In experiment 1, there were 5 treatment levels, but in experiments 2 and 3, there were 4 treatment levels. We did a separate behavioral trial in which the highest concentration was 25%. During exposure, the salinity (30.9 to 31.1 ppt), pH (6.6 to 6.8) and dissolved O_2 (4.35 - 4.5 mg/l) in the different treatments was within the narrow range of those seen in the 0.2SW used as a control, however the pH and dissolved O_2 was at the lower end of this range in the 10% PW treatments.

After Exposure

After exposure, the larval were transferred into beakers containing a 105µm mesh. We used the sunken filter technique (Strathmann 1987) at all times so as not to expose larvae to air. We flushed all beakers at least twice with 0.2SW to remove traces of PW. Larvae were transferred from exposure in the same order in which they were added. After flushing, larvae from each beaker were carefully washed into 0.2SW in 15ml Petri dishes and stored. When all larvae had been flushed and transferred (generally a 10-15 minute process), they were transferred individually into growth beakers.

Growth beakers were 10ml disposable plastic beakers (Fisher Scientific) filled with ~8ml of 0.2SW and contained only one larva each. The larval behavior (see "*Behavioral Endpoints"*) was noted within the first hours and at a number of times while being grown in the lab. Larvae were fed a 1ml mixture of phytoplankton (*Isochyris* and *Rhodomonas*) after they settled and water was changed at least every other day.

Field Outplanting and Monitoring

At outplanting, the colonies were generally only the ancestrula plus some of the first zooid. We gently cut out the plastic beaker around the colonies and glued it a PVC back-board (sizes of the boards ranged up to 75cm x 36 cm depending on the space required in each experiment), which was hung from floating docks in the Santa Cruz Harbor.

For experiments 2 and 3 and days 110 and 150 only of experiment 1 (see Table 1 for details), the outplanted adults were assessed using a camcorder (Sony Hi-8 TR 400: x12 optical zoom) with close up filters (total possible magnification ~ x20). The video images were captured in the lab and analyzed using NIH Image for area, number of zooids and perimeter of colony (NIH Image is a public domain program developed at the US National Institutes of Health and available at http://rsb.info.nib.gov/nih-image/). For all census times before day 110 in experiment 1, colonies were counted using a field microscope. No data about colony size were taken at these census times.

Table 1. A summary of the experimental conditions for *W. subtorquata*

Experiment, Start & Exposure Batches	**Exposure Duration**	**Concentrations**	**Outplanted**	**Census Days**	**Larvae**
1: November 1997 (No Batches)	50 mins	0%, 0.01%, 0.1%, 1%, 10%	After 8 Days For 150 Days	Day 10,20, 30, 39, 81, 110, 150	89 total 70 used
2: February 1998 (No Batches)	50 mins	0%, 0.1%, 1%, 10%	After 8 Days For 80 Days	Day 20, 25 40, 60, 80	46 total 39 used
3: August 1998 (4 Batches)	65 mins	0%, 0.1%, 1%, 10%	After 12 Days For 40 Days	Day 10, 20, 40	323 total 192 used

Behavioral Endpoints

At each stage, we measured mortality and any visible abnormalities in the larvae or adults. Following are the endpoints for each phase of the experiments with *W. subtorquata* but many are common to the experiments with other larvae.

During Exposure

- The swimming behaviour of the larvae. This was assessed as either swimming or not. Other experiments ran parallel to the exposure experiments to quantify larval swimming (see "Behaviour Trial").
- The number of larvae settled.

After Exposure

In this phase, larval behavior was placed into one of 7 categories:

- Swimming;
- Searching - temporarily attached;
- Not moving;
- Metamorphosed;
- Operculum visible (in later counts, this became the number of opercula visible);
- Dead (clearly dead with a evidence remaining);
- and unknown (this included larvae that disappeared).

Some of the categories were pooled for analysis (e.g., the categories: 'searching - temporarily attached' and 'metamorphosed' are both a part of the settlement process and were often lumped together as 'settling'.

We measured:

- Larval behaviour soon after exposure (within <1 to 3 hours).
- Larval behaviour one day (24 hours) after exposure.
- Larval behaviour at various times during the lab growth phase (details are shown where data are reported).

Field Outplanting and Monitoring

The colony size was measured as both the number of zooids and zooid size (mm^2). We measured a number of endpoints.

- The number of zooids at outplanting.
- The adult growth was measured as the change in the number of zooids between census dates (see Table 1 for census details).
- The zooid size (average zooid size in per mm^2) was also calculated for each census date. This measure of colony size allows the zooids counts to be scaled for differential growth in the colonies by taking into account the area the colony occupies.
- The competitive ability of the colonies. This experiment was only attempted with *Watersipora subtorquata.* The competitive load on the colonies was assessed for each treatment. Competitive ability was measured as the ability of colonies to maintain themselves against competing neighbours ("draws"), the inability to withstand overgrowth by neighbours ("losses"), or the ability to overgrow neighbours ("wins"). A drawn situation is a

stalemate where neither the target colony nor the neighbour has the upper hand and there is a change in growth pattern of both competitors. A loss is when the target colony has been overgrown, whereas in a win the target colony has overgrown its neighbours. From a *W. subtorquata* colony perspective, these situations are not independent interactions as most *W. subtorquata* colonies will experience at least two of these conditions at one time. A loss results in the reduction of biomass and hence potential reproductive output due to overgrowth, and should be more detrimental to a colony than a draw. In a loss situation, we estimated the proportion of total area overgrown on the target colony. This was done on the computer screen with captured images by predicting the growth pattern in the absence of the ascidian based on the growth at this and previous census dates. It is an estimate as it is hard to define exactly where a colony is underneath a competitor. However, due to the controlled nature of the surface in this experiment, even after 150 days the colonies were quite regularly shaped. The competitors that were in large enough numbers to analyse were colonial ascidians of two genera: *Botryllus* and *Botrylliodes*.

Analyses

Mortality and behavior

To analyze most behaviors and mortality, we used hierarchical, log linear modeling with batch, PW concentration (treatment) and the behavior as categorical variables (Sokal and Rohlf 1995). Initially, a model is fitted with all the interaction terms and used to calculate the G^2 statistic. To find the significance of each term, you remove that term, re-run the model and calculate a ΔG^2, which is compared to a distribution similar to the χ^2 distribution.

In Experiment 3, we exposed larvae to PW in four artificially allotted batches as opposed to the pooled exposure in experiments 1 and 2. We tested for an effect of batch on larval reactions to the treatments at various stages:

- The swimming behaviour of the larvae 1 hour after being removed from PW.
- The larval metamorphosis 24 hours after being removed from PW.
- The mortality 24 hours after being removed from PW.
- The number of colonies with opercula by Day 4.
- The mortality at outplanting.
- The size of colonies at outplanting.
- Mortality of the colonies at Day 10, Day 20 and Day 40.
- the size of the colonies at Day 10, Day 20 and Day 40.

Of particular interested are any batch x treatment effects on larval behaviors: swimming, settled/metamorphosed, operculum development and survival. Some of the interactions are biologically meaningless and so we have not included them in the results. We have detailed the results for the batch x treatment x "behavior", treatment x "behavior" and batch x "behavior". If there is a significant batch x "behavior" interaction, it simply shows that larvae in different batches had different behaviors, regardless of treatment. This interaction may be biologically interesting but is not important in the context of these experiments as we are only interested in interactions of behaviors with the treatments. A significant batch x treatment x "behavior" indicates that the difference in behavior with treatment depends on the batch of larvae used. When there was a batch x treatment x 'behaviour' effect, we checked the frequencies for a

pattern. If there was a clear pattern, we removed that batch and re-ran the analysis. As this is technically an unplanned comparison (Sokal and Rohlf, 1995), we corrected the alpha level for the unplanned test. Generally there was only one extra test and hence we I used an error rate of $\alpha = 0.025$. This is a very conservative error correction (Sokal and Rohlf, 1995).

A significant treatment x "behavior" indicates that the larval behavior differs between treatments. To ascertain which treatments were important, we made 3 planned comparisons using the Fisher's Exact χ^2 or the Yate's corrected χ^2, whichever was appropriate (Sokal and Rohlf, 1995).

Growth and competitive ability

All data measuring growth and competitive ability of adults or larvae were analyzed using variations of ANOVA. Where necessary the data were transformed to maintain homogeneity of variances and normality (Underwood 1997). We analyzed the growth data with a repeated measures ANOVA where growth was the repeated measure (assessed as the change in number of zooids between census dates).

The data in experiments 1 and 2 were analyzed using ANOVA with Treatment (fixed factor; 4 or 5 levels) as the single factor. The data in Experiment 3 were analyzed using 2-way ANOVA with Batch (4 levels) and Treatment (4 levels) as fixed factors. The treatment effects in this experiment were compared between batches first and if no Batch x Treatment interaction was found, the data were pooled across batch for further analysis of the treatment effect. For each significant treatment effect, we compared the control to each treatment level in a pairwise comparison using a two-sided Dunnett test (Underwood 1997) as this was the most biologically interesting. Some marginally non-significant results with low power were also tested using this method.

Due to low sample sizes in some experiments possibly resulting in increased Type II errors, we checked the power of all non-significant tests to pick up a change using Pass 6.0 (www.ncss.com/pass). We based the effect size on natural levels of variation in this system and the alpha level was set at 0.05.

Behavioral Trial

We ran one experiment separately from the exposure-outplant experiments to detail the behavior of the larvae during exposure. The larvae used in the behavior trial came from a subset of the batch of adults used in Experiment 3 and the trial started the day before the release of larvae for Experiment 3.

We did the behavior trial in 20 ml disposable plastic beakers (Fisher Scientific) filled with at least 10 ml of 0.2SW and PW. In this trial, there were 5 treatments: 0%PW, 0.1%PW, 1%PW, 10%PW and 25%PW with 5 replicates of each. We used 186 larvae placing multiple larvae in each beaker (4-8 larvae per beaker) and calculated the % swimming after 15, 30, 45, 75 minutes and 23 hours. We also noted if any larvae attached or metamorphosed. These larvae were never washed from the PW and never outplanted. These data did not require analysis for the swimming behaviors at 15 and 75 minutes, however we used a 1-way ANOVA to analyze the proportion

attached after 75 minutes and a two-sided Dunnett test to compare between treatments. After 23 hours of constant exposure, we analyzed the proportion of larvae metamorphosed in each treatment with a similar one-way ANOVA and two-sided Dunnett test.

For extra behavioral information we also quantified the swimming behavior of the larvae used in Experiment 3 during the first 10 minutes of exposure and up to 75 minutes of being transferred to clean 0.2SW. There were 4 replicate batches of each treatment in this test with between 14 and 25 larvae in each. The analysis was the same as the main behavioral trial but data were analyzed for the first 5 to 10 minutes during exposure and at 75 minutes after exposure only.

Schizoporella unicornis

We ran one experiment in June 1998 with methods similar to those used for *Watersipora subtorquata*. The larvae were raised in the lab for 17 days after exposure to the 4 standard PW concentrations (Boxshall and Raimondi, *in prep. B*). Larval development was followed in parallel studies with similar behavioral endpoints measured as with *W. subtorquata*. The colony size was measured at outplanting. There was extraordinary growth on the outplanted boards for this experiment, which obscured much further analysis. Analyses were the same as those used above.

Bugula neritina

We ran a series of experiments in late 1999 using the larvae of the upright bryozoan, *Bugula neritina* (Boxshall and Raimondi, *in prep. B*). The general methods were very similar to those used for *W. subtorquata.* One important difference is in the method for assessing the size of the colony. As *B. neritina* have an upright growth form, the standard way to assess growth is to count the number of bifurcations in the colony (Keough 1989). The size of the colonies was measured at outplanting and at Day 70 when the experiment ended. Another difference was that the number of ovicells present on the colonies was counted at the end of the experiment. For analysis, the number of ovicells was scaled for size of the colony (i.e., number of bifurcations) and analyzed as $\log_{10}$ (ovicell density+1). This enables some estimate of reproductive differences between treatments. *B. neritina* were batched for all experiments. There were no differences between batches that affected the treatments (Boxshall, unpubl. data).

***Botrylloides* spp.**

We ran a series of experiments in mid 1998 using the larvae of the colonical ascidian, *Botrylloides* spp. The general methods were very similar to those used for *W. subtorquata.* These experiments were not successful as the larvae only swim for approximately 20 minutes before settling. We obtained preliminary data on the swimming behavior of *Botrylloides* spp (Boxshall, unpubl. data).

Asterina miniata
The behaviors of these larvae to a biofilm cue were far less specific than we had hoped, making them untractable for use in PW experiments. Although an extensive series of pilot tests were complete, we were unable to find a tractable, cued surface to which this larvae would attach. *Asterina miniata* show very interesting swimming behaviors and would be very useful for future work into the detailed impacts of PW on swimming behaviors of invertebrates.

Haliotis rufescens
The abalone larvae were tested for settlement ability as competent larvae after exposure to four treatments of PW in 0.2SW (0%, 0.01%, 0.1% and 10%) for one hour. As a cue to settlement, 10^{-6} GABA was used. The proportion of larvae settled in 20ml disposable beakers was assessed and compared to control beakers without GABA. The proportion of larvae settled was tested using a two-way ANOVA (Treatment: 4 levels; Cue: 2 levels). Data did not require transformation.

Results

General Comments

This results section is a summary of many of the important results from the project. Further detailed results and analyses are to be published in a series of papers (Boxshall and Raimondi, *in prep A, B and C*).

Specific Results

Watersipora subtorquata
<u>Batched Exposure vs Pooled Exposure</u>
In summary there was no effect of batching the larvae during exposure on larval activities very early in life (i.e., swimming (table 2), metamorphosis at 23 hours (table 3), survival in the first 24 hours (out of 185 larvae, only one had clearly died and one other had disappeared from different batches) or later as adults (growth at outplanting, and both growth and survival at Days 10 and 40 (table 4)). However, there was a difference b/n batches in the development of opercula (Day 4) in the lab and the survival of juveniles to outplanting (table 5 and 6). These are early stages of development, but after metamorphosis.

Table 2: *W. subtorquata* swimming behaviors. Swimming 75 minutes after being washed from PW.

	G^2	df	*p*	ΔG^2	df	*p*
batch x tmt x sw (full model)	9.2970	9	0.410			
treatment x sw	15.77	12	0.202	6.47	3	0.091
batch x sw	17.47	12	0.133	8.17	3	**0.043**

Table 3: *W. subtorquata* larval metamorphosis 23 hours after being washed from PW.

	G^2	df	*p*	ΔG^2	df	*p*
batch x tmt x met (full model)	10.4019	9	0.319			
treatment x met	26.38	12	0.010	15.98	3	**0.001**
batch x met	13.31	12	0.347	2.90	3	0.407

0% Vs 0.1% (i.e., without 1% and 10%)

	G^2	df	*p*	ΔG^2	df	*p*
batch x tmt x met (full model)	1.375	3	0.771			
treatment x met	10.18	4	0.037	8.81	1	**0.003**
batch x met	5.33	6	0.502	3.96	3	0.266

0% Vs 10% (i.e., without 0.1% and 1%)

	G^2	df	*p*	ΔG^2	df	*p*
batch x tmt x met (full model)	4.142	3	0.247			
tmt x met	12.85	4	0.012	8.71	1	**0.003**
batch x met	4.46	6	0.614	0.32	3	0.956

Table 4: Survival of *W. subtorquata* colonies to the final day in Experiment 3 (Day 40).

	G^2	df	*p*	ΔG^2	df	*p*
batch x tmt x surv (full model)	11.195	9	0.263			
tmt x surv	13.39	12	0.342	2.19	3	0.533
batch x surv	11.58	12	0.480	0.39	3	0.943

Table 5: *W. subtorquata* survivorship to outplanting in Experiment 3, comparing the batches. We have included mean survival for comparison.

Treatment	**% Total Survival**				
	Mean	Batch 1	Batch 2	Batch 3	Batch 4
0%	98	92	100	100	100
0.1%	95	100	100	100	80
1%	87	73	92	82	100
10%	84	92	83	92	67

Table 6: *W. subtorquata* survivorship to outplanting in Experiment 3.

	G^2	df	*p*	ΔG^2	df	*p*
batch x tmt x surv (full model)	**17.415**	**9**	**0.043**			
tmt x surv	24.06	12	0.020	6.64	3	*0.084*
batch x surv	19.32	12	0.081	1.91	3	0.592

Re-analyzed without Batch 4. Note an alpha level of 0.025 this analysis.

	G^2	df	*p*	ΔG^2	df	*p*
batch x tmt x surv (full model)	**6.478**	**6**	**0.371**			
tmt x surv	**17.65**	**9**	**0.039**	**11.18**	**3**	**0.011**
batch x surv	7.95	8	0.439	1.47	2	0.479

Re-analyzed survival as planned comparison s without Batch 4 to test which tmt differed from the control.

	Pearson χ^2	df	*p*	Fisher's Exact (*p*)
0 % vs 10 %	1.934	1	0.354[#]	*0.357
0% vs 1%	8.016	1	**0.013**[#]	***0.006**
0% vs 0.1% ^				

[#] We have used a corrected alpha level of 0.017 to test the null hypotheses due to previous comparisons of these data.

* Due to small number of frequencies in some cells, I have used the conservative Yate's corrected χ^2 in the 1% and 10% comparison (Sokal and Rohlf, 1981). I have also shown the Fisher's Exact test *p* for comparison.

^ There is no difference in survival between batches 1,2 and 3 for this treatment.

Behaviour during and after exposure

The larvae consistently swam less (Figure 1), settled less (Table 7) and showed less movement of any kind under the influence of higher concentrations of PW (Boxshall, unpubl. data). Not surprisingly, the 25% PW impacted heavily on larvae (table 7). There was little difference in the activity of larvae within hours of exposure to PW if they were washed in clean, filtered seawater (Table 8).

After 24 hours, most larvae ×± sd : 72 ± 18%) had metamorphosed and there was no difference between treatments (df= 3, G^2 = 4.96, *p*= 0.176). By day 3 (66 ± 12%) or day 4 (77 ± 8%) the number of colonies with opercula also did not differ between treatments (Day 3: df= 3, G^2 =1.49, *p*= 0.684; Day 4: df= 3, G^2 = 0.86, *p*= 0.836).

There was no difference between all the treatments for the number of colonies that had developed opercula by either 3 (× ± sd : 73 ± 7%) or 4 (86 ± 6%) days after exposure (Day 3: df= 4, G^2 =1.42, *p*= 0.840; Day 4: df= 4, G^2 = 1.76, *p*= 0.779).

Table 7.

A. The proportion of *W. subtorquata* larvae attached (settling) after 75 minutes of exposure. These data did not require transformation.

Source	**df**	**MS**	**F-ratio**	***p***
Treatment	4	0.249	12.808	**0.000**
Error		20	0.019	

Two-sided Dunnett test

Tmt	**Mean differences from control**	***p***
0.1%	-0.018	0.999
1%	-0.159	0.245
10%	-0.449	**0.000**
25%	-0.452	**0.000**

B. The proportion of *W. subtorquata* larvae metamorphosed after 23 hours of constant exposure. These data were arcsine transformed due to the high number of cells with a value of 100%.

Source	**df**	**MS**	**F-ratio**	***p***
Tmt	4	1.207	12.722	**0.000**
Error	20	0.095		

Two-sided Dunnett test

Tmt	**Mean differences from control**	***p***
0.1%	-0.024	1.000
1%	0.043	0.998
10%	-0.257	0.501
25%	-1.127	**0.000**

Figure 1. Proportion of *Watersipora subtorquata* swimming during exposure to PW after 15 minutes and 75 minutes.

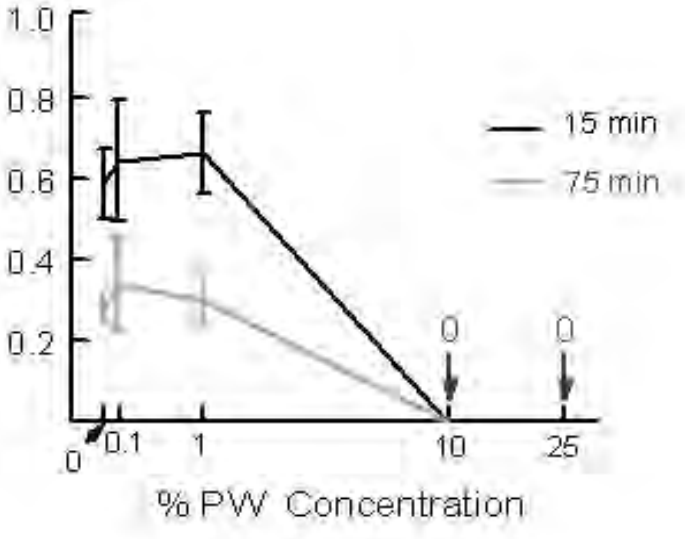


Table 8. The percentage of *W. subtorquata* larvae showing each behavior 3 and 31 hours after exposure. The numbers in brackets are the actual number of each larvae showing each behavior.

Treat-ment	Percent Post-exposure behavior								
	Percent 3 hours after exposure					Percent 31 hours after exposure			
	total larvae	swim	settlement activities	not moving	dead	swim	settlement activities	not moving	dead
0%	**10**	10 (1)	90 (9)				100 (10)		
0.01%	**15**		80 (12)	7 (1)	13 (2)		87 (13)		13 (2)
0.1%	**15**	13 (2)	80 (12)		7 (1)		87 (13)		13 (2)
1%	**15**	7 (1)	73 (11)	20 (3)			93 (14)		7 (1)
10%	**15**		67 (10)	20 (3)	13 (2)		80 (12)		20 (3)

Mortality

When larval mortality occurred, it tended to be in higher concentrations of PW and was visible after the first hours of exposure rather than during exposure. There was no mortality during exposure in Experiments 1, 2 or 3.

In Experiment 1, there was no mortality at all in the control (0%) during the laboratory phase of this experiment. What mortality that did occur was in PW treatments. It was not possible to test these data due to small sample sizes (low mortality in >1/3 of the cells). However, there was little mortality and very little difference between treatments with PW at any census time in the lab. At outplanting, there had been a uniform 20% mortality in all the PW treatments.

Most mortality in the field occurred between Day 39 and Day 81. However by day 81there was no difference in mortality between the treatments (df=4 , G^2 =1.82 , *p*=0.768). By Day 150, there was no difference in mortality between the treatments (table 3; df=4 , G^2 =2.04 , *p*=0.728) but note that the there was more mortality in the 10% treatment than in the 0%. The highest survivorship of colonies that were outplanted was in the 1% treatment.

In Experiment 2, there was no difference in mortality between the treatments 15 minutes after exposure (only 3 larvae dead: 2 in 0.1%, 1 in 1%), 24 hours after exposure (total of 4 larvae dead: 3 in 0.1%, 1 in 1%) or 8 days after exposure, prior to outplanting (df= 3, G^2 = 1.27, *p*= 0.736). The small numbers in this experiment meant that testing for mortality differences was generally not possible (low mortality in >1/3 of the cells). There was little difference in mortality as the colonies grew in the field. The only sizeable mortality occurred in the 10% treatment and mainly between Day 40 and Day 60. In experiment 2, the highest mortality was in the 10% treatment and the highest survivorship was the 1% treatment, as in experiment 1.

In experiment 3, there was little early mortality in this experiment in the lab. There was no mortality within an hour of transferring the larvae from PW to clean 0.2SW. The mortality 24 hours after being removed from PW was very low. Two larvae out of 185 were dead or missing. There was no batch x treatment effect on early mortality.

There was increased mortality at outplanting in treatments with higher PW concentrations. However, there was no clear pattern amongst batches. There was a significant batch x treatment x survival interaction but the reasons are not that clear (Table 6). Batch 4 seemed to be driving that result, as evidenced by the lack of batch x treatment x survival interaction in the analysis without batch 4 (table 6). When batches 1,2 and 3 were pooled and re-analyzed, there was no statistical difference in survival between the control and 10 % treatment, however there was statistically greater mortality in the 1% treatment than in the control (table 6).

There was very little difference in mortality of the colonies at day 10 or day 20, between the treatments or within the batches in each treatment and no statistical differences at day 40. At days 10 and 20 it was not possible to test for a batch x treatment interaction, or even treatment effects as not enough larvae had died. Only 8 larvae out of 128 were dead at day 10 (15/128 at day 20). There was little clear pattern to this slight mortality, but the mortality in the 10% treatment was always greater than the control. Most mortality occurred from day 20 to day 40. When the mortality from day 20 to day 40 was compared, there was no difference in survival between treatments or within batches (Table 9).

Table 9. Experiment 3. *W. subtorquata* survival from Day 20 to Day 40.

	G^2	df	*p*	ΔG^2	df	*p*
batch x tmt x surv (full model)	14.460	9	0.107			
tmt x surv	16.22	12	0.181	1.76	3	0.624
batch x surv	14.55	12	0.267	0.09	3	0.993

Growth

In experiment 1, the colonies were the same size in all treatments at outplanting ($F_{4,53}$ =1.040, *p*=0.396), but given the variability in the sizes, the power was low (26%). From outplanting to day 150, the growth trajectory was similar between all treatments (figure 2, Table 10). There was no effect of PW exposure at all on the growth rates over time.

Table 10. Experiment 1. Repeated Measures of *W. subtorquata* growth rates from Day 0 to Day 150. Given the p values, I have only included the univariate results and given the epsilon values, we have included the GG corrected p-values.

Between Subjects

Source	**df**	**MS**	**F-ratio**	***p***
Tmt	4	0.468	2.026	0.120
Error	26	0.231		

Within Subjects

Source	**df**	**MS**	**F-ratio**	***p***	**G-G**
Time	6	11.787	76.591	0.000	0.000
Time*Tmt	24	0.086	0.557	0.953	0.804
Error	156	0.154			
Greenhouse-Geisser Epsilon:		0.3261			
Huynh-Feldt Epsilon:		0.4066			

The final size of the colonies, measured simply as the number of zooids, was not different between treatments at day 150 ($F_{4,30}$ = 1.320, *p*=0.285), however the power in this test was low (32%) and there is a trend for smaller final size in the 10 % PW. The average final size of the zooids at Day 150 was not different between treatments (table 11, figure 3). As the result was marginal at an α =0.05 level (*p*=0.061) and the power was low (power=58%), we ran a pairwise comparison of the PW treatments with the control. There was no difference between any of the treatments and the control (table 19). Due *a priori* decisions, we only tested the PW concentrations against the control. Constrained by degrees of freedom, we can only speculate that the effects of exposure may be stronger at intermediate concentrations (Figure 3).

Table 11. The size of *W. subtorquata* zooids in Experiment 1 at day 150. These data did not require transformation.

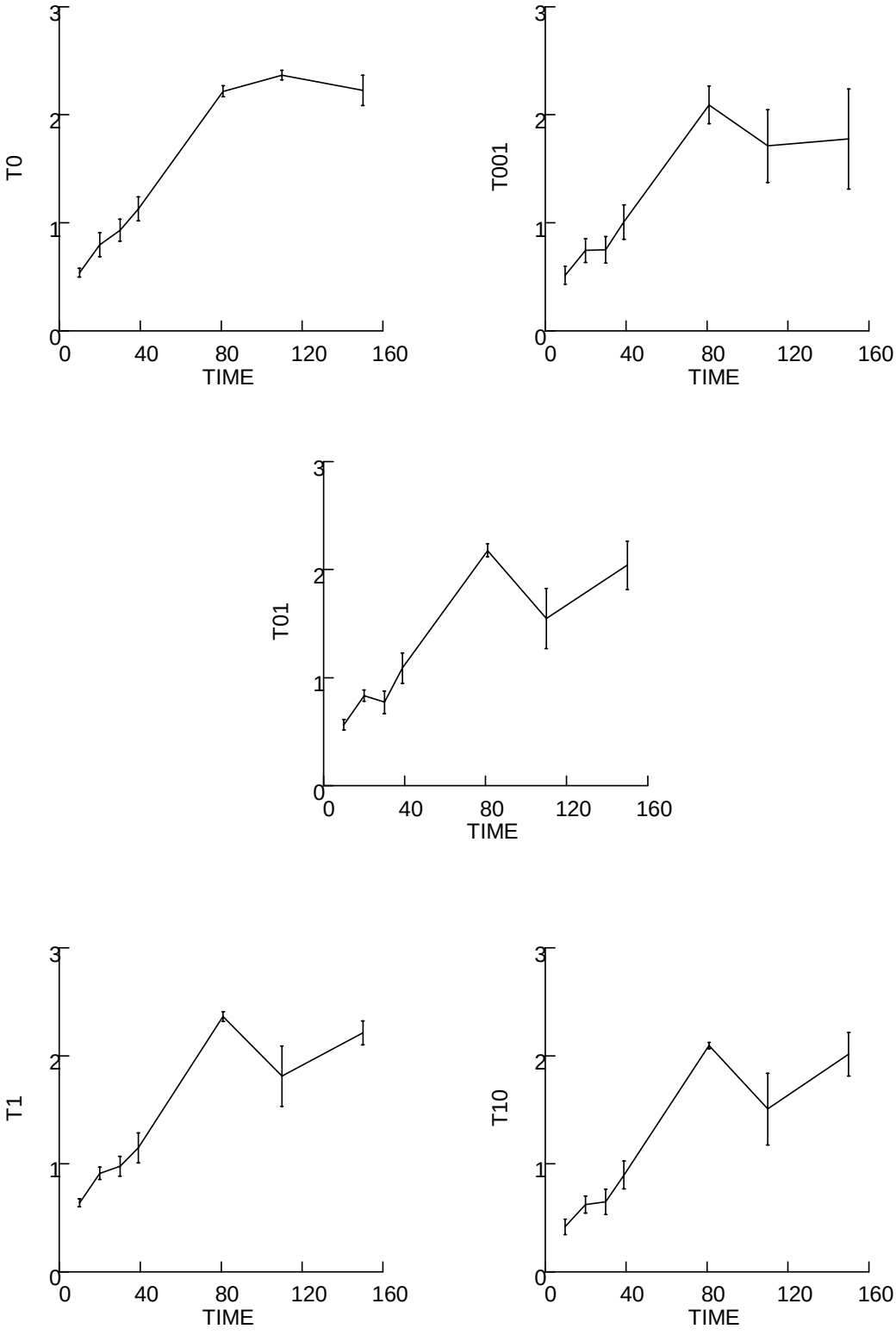
Source	**df**	**MS**	**F-ratio**	***p***
Treatment	4	0.572	2.541	0.061
Error	29	0.225		

Two Sided Dunnett Test

Tmt	**Mean differences from control**	***p***
0.01%	-0.277	0.696
0.1%	0.439	0.292
1%	0.288	0.598
10%	-0.106	0.984

In experiment 2, all the colonies surviving to outplanting were the same size ($F_{3,34}$ =0.242, p=0.867), as were the subset chosen to ouplant ($F_{3,22}$ =0.850, p=0.482). From outplanting to Day 80, there was no difference in the growth trajectory due to the treatments (table 12). The final size of the colonies (day 80), measured as the number of zooids ×± se : 521 ± 50) was not different between treatments ($F_{3,17}$ =0.321, p=0.810). Nor was the average size of the zooids (× ± se:0.617 ± 0.03 mm^2) different between treatments at day 80 ($F_{3,22}$ =0.282, p=0.838).

Figure 2. Experiment 1. Adult growth in the field for *W. subtorquata* over the entire duration of the experiment. Growth is measured in Log_{10} (growth+1), where growth = the number of zooids added between census dates. T0 to T10 represent the different PW treatments and time is measured in days.



In experiment 3, all colonies were statistically the same size (measured as the number of zooids) at outplanting. There was no interaction with, or difference due to the main effect of, batch when the colonies were outplanted (Table 13). Also colonies had a similar number of zooids regardless of treatment.

From outplanting to Day 40, there was no difference in the growth trajectory for any treatment or due to any batch x treatment interactions (Table14). The average size of zooids at day 40 differed between treatments. At the alpha = 0.05 level, there was no batch x treatment interaction, however there was a significant effect of treatment (Table 15). The test of batch x treatment (p=0.056) has a power of 100% so we are comfortable there was no batch x treatment effect at an alpha = 0.05 level. There is no consistent pattern amongst treatments. I pooled the batches and have analyzed the zooid size between the treatments (table 15). There is a statistical difference between some of the treatments however none are different to the control.

Figure 3. Experiment 1. The final size of *W. subtorquata* colonies at Day 150. Both axes are displayed as logs.

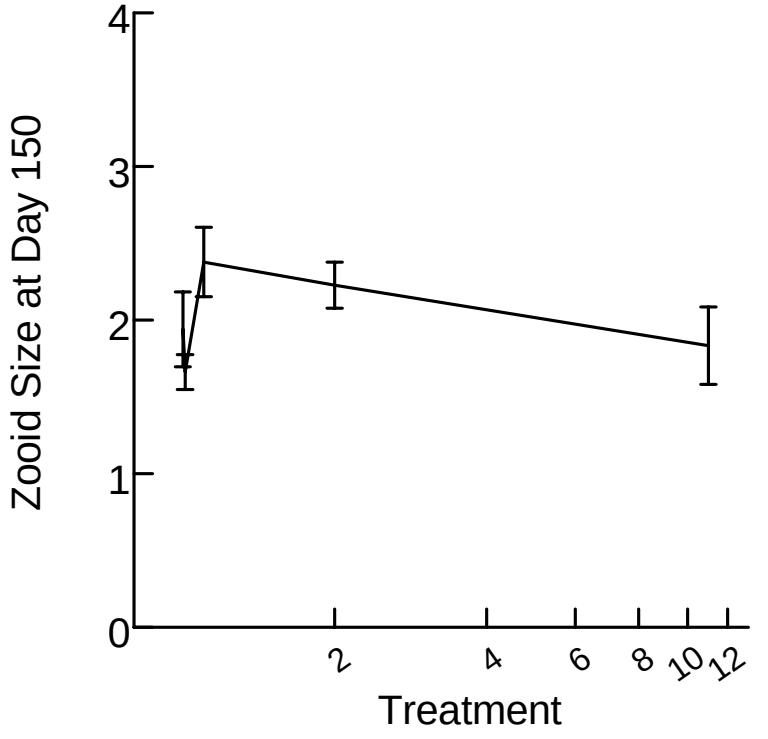


Table 12. Growth rates of *W. subtorquata* throughout Experiment 2. The univariate results from a repeated measures ANOVA. These data have been $\log_{10}$ transformed.

Within Subjects	**df**	**MS**	**F-ratio**	***p***	**G-G**
Growth	4	10.815	505.340	**0.000**	**0.000**
Growth x Tmt	12	0.022	1.021	0.445	0.439
Error	48	0.021			
Greenhouse-Geisser ξ	0.608				
Huynh-Feldt ξ:	0.964				

Table 13. The size of *W. subtorquata* colonies in Experiment 3 at outplanting.

Variable	**df**	**MS**	**F-ratio**	***p***
Batch	3	0.104	0.529	0.663
Tmt	3	0.063	0.322	0.810
Batch*Tmt	90.169	0.858	0.564	
Error	149	0.197		

Table 14. Growth rates of *W. subtorquata* throughout Experiment 3. This repeated measures reports the unadjusted *p*-values as the G-G and H-F ξ values are 1 or close to it .

Within Subjects	**df**	**MS**	**F-ratio**	***p***
Growth	2	37.294	922.053	**0.000**
Growth x Batch	6	0.057	1.421	0.211
Growth x Tmt	6	0.039	0.975	0.445
Growth x Batch x Tmt	18	0.049	1.223	0.251
Error	134	0.040		
Greenhouse-Geisser ξ:	0.982			
Huynh-Feldt ξ:	1.000			

Table 15

A. Experiment 3. Average *W. subtorquata* zooid size (log_{10}(zooid size+1)) at the final census date (Day 40). This test has a power of 100%.

Source	**df**	**MS**	**F-ratio**	***p***
Batch	3	0.001	1.518	0.217
Tmt	**3**	0.006	5.843	**0.001**
Batch x Tmt	9	0.002	1.973	**0.056***
Error	69	0.001		

B. Experiment 3. Average *W. subtorquata* zooid size (log_{10}(zooid size+1)) at the final census date (Day 40) pooled across batches.

Source	**df**	**MS**	**F-ratio**	***p***
Tmt	**3**	**0.007**	**6.485**	**0.001**
Error	81	0.001		

Two Sided Dunnett Test

Tmt	**Mean differences from control**	***p***
0.1%	-0.020	0.117
1%	0.022	0.062
10%	0.008	0.795

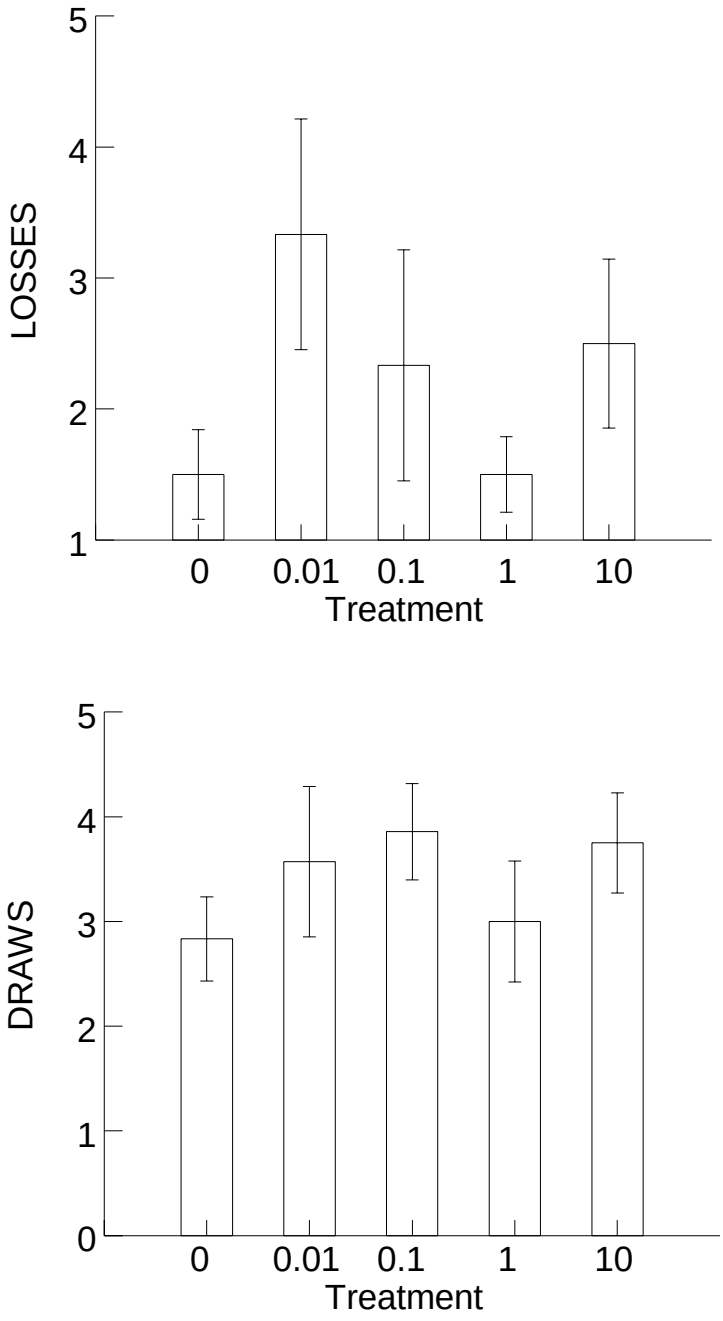
Competitive ability

In experiment 1, we could identify a total of 7 deaths in the entire experiment due directly to overgrowth. There was no real pattern, with each treatment represented once except for 3 from 0.1%. At day 150, every colony except one was interacting on average with at least 3 colonial ascidians. There was no difference in the number of ascidians ($\times\pm$ se : 4.3 ± 0.4) interacting with target colonies across treatments ($F_{4,31}$ = 0.744, p=0.569) although there was only 17.5% power. This measure can be considered a crude indicator of the overall competitive load on the colonies due to interactions with colonial ascidians. There were never situations where the *W. subtorquata* colony clearly overgrew the ascidian (a "win"). Almost every colony was involved in a "draw" with multiple ascidian competitors but there is no clear pattern from these data ($F_{4,26}$ = 0.648, p=0.633; Figure 4). Most colonies had a "loss" but again there was no clear pattern across treatments ($F_{4,13}$ = 1.263, p=0.334, power = 12%; Figure 4). There was no difference in the proportion of estimated area lost through overgrowth ($F_{4,14}$ = 0.728, p=0.587) and, again, the power was low (17%). These data were log_{10} transformed to maintain normality for analysis.

Of the 21 colonies remaining in experiment 2 at day 80, ten were interacting with neighboring colonial ascidians however there was no clear pattern from these interactions. Eleven colonies had no neighbors at the time of census, including all remaining colonies from the 10% treatment. No colonies were being overgrown by colonial ascidians (Loss), 3 were overgrowing colonial ascidians (Wins: 1 x 0%, 2 x 1%) and 7 had 1 or more colonial ascidians touching them without a clear overgrowth by either the bryozoan or ascidian (Draws). The only colonies that clearly died from overgrowth were both from the 10% treatment (1 at Day 40, 1 at Day 80). Overall,

again there was no clear decrease in competitive abilities across treatments. Due to the shorter duration of experiment 3, there were not enough competitive interactions for analysis.

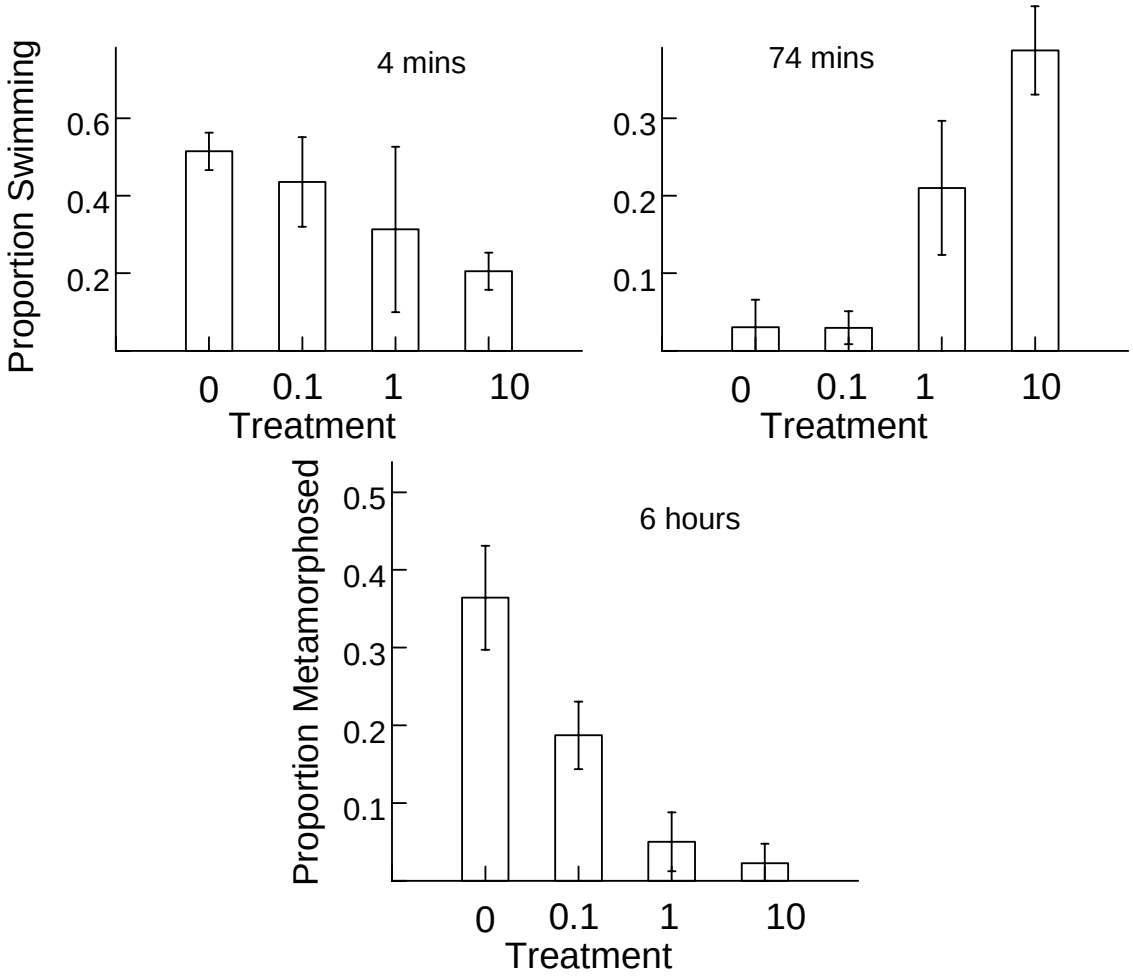
Figure 4. Competitive interactions of *W. subtorquata* with colonial ascidians. Note the difference in scales. For details of what constitutes 'Losses' and 'Draws', see text.



Schizoporella unicornis

The *Schizoporella unicornis* larvae showed an interesting pattern similar to *W. subtorquata* larvae. After four minutes of exposure to PW, the trend was for larvae to be swimming more in the lower PW concentrations. Those not swimming were searching on the surface or not moving. More were in the "not moving" category at 10% PW concentration (Figure 5). After 74 minutes of exposure, there were more larvae swimming in the higher concentrations. However, at this time, those in the lower concentrations had started to attach and metamorphose. By 6 hours of exposure, many more *S. unicornis* larvae had metamorphosed in the control than any other PW treatment, especially when compared to 10% PW (figure 5). Mortality was negligible during this exposure test, and after exposure in other tests while larvae were in the laboratory.

Figure 5. The proportion of *S. unicornis* larvae swimming and metamorphosed at different times after exposure to PW. Note the different scales.

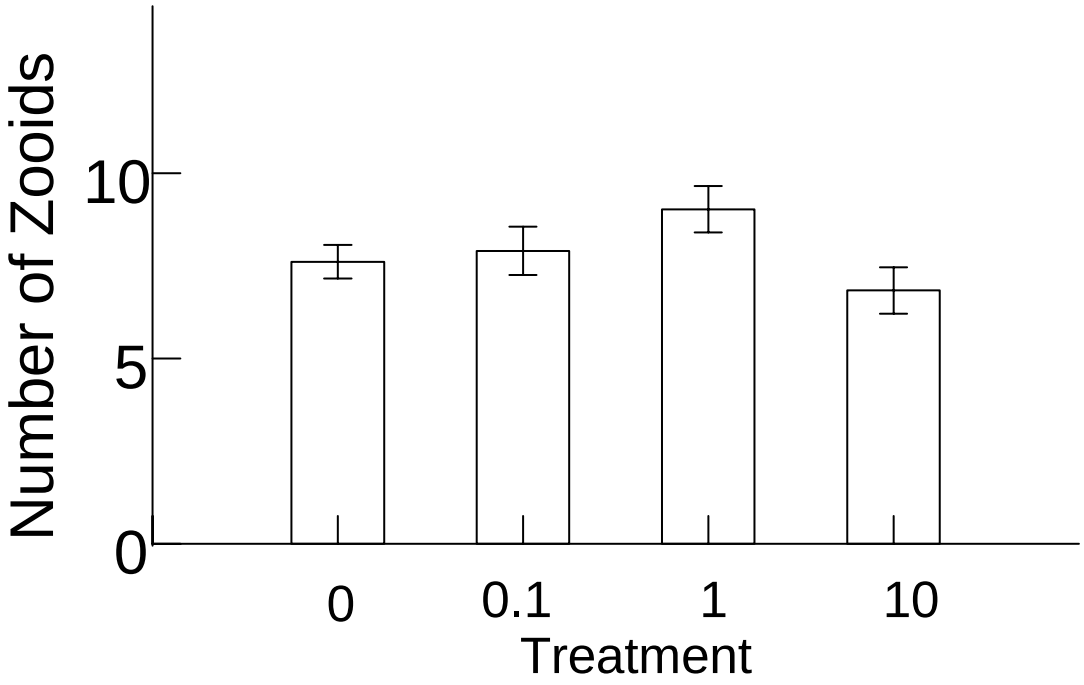


By the time for outplanting the *S. unicornis* colonies, there was no difference in the size of the colonies as measured by the number of zooids, between the treatments. There was no difference between the four batches of larvae, however there was a trend for the colonies in 10% PW to be smaller than the other colonies (Table 16, Figure 6).

Table 16. The size of *S.unicornis* colonies at outplanting to the field.

Source	**df**	**MS**	**F-ratio**	***p***
Batch	3	9.093	0.865	0.461
Tmt	3	25.870	2.462	0.066
Batch x Tmt	9	11.891	1.132	0.347
Error	109	10.509		

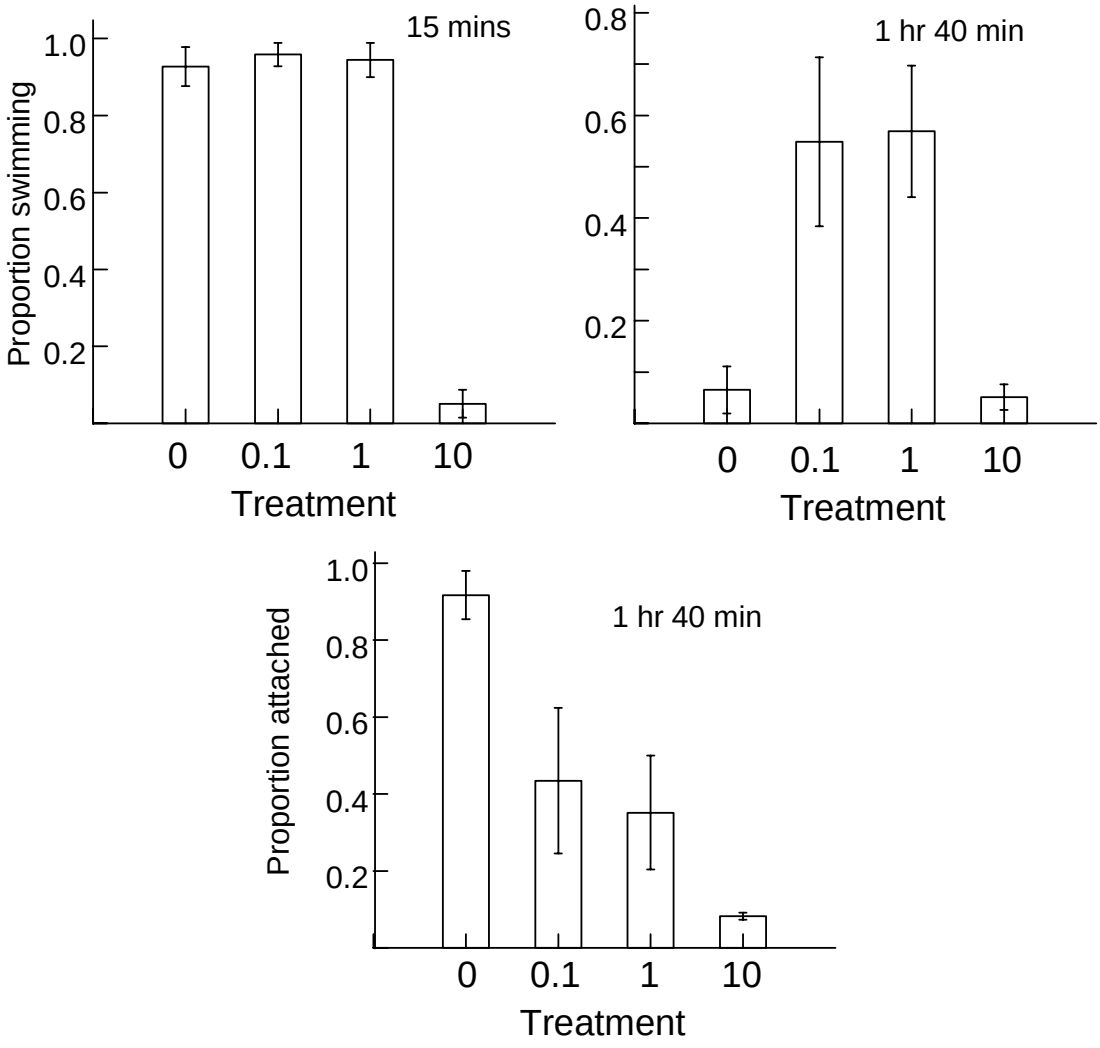
Figure 6. The size of *Schizoporella unicornis* colonies at outplanting.



Bugula neritina

After 15 minutes of exposure to PW, almost all the larvae in the 0%, 0.1% and1% treatments were swimming, compared to <10% of larvae in the 10% PW treatment. After 1 hour and 40 minutes of exposure, most larvae were still swimming in the 0.1% and1% treatments but most of the larvae in the control had metamorphosed. This was not the case for the 10% PW treatment. Most larvae were not moving (figure 7). Mortality was negligible while larvae were being exposed to PW. This results was mirrored in the parallel experiment which exposed larvae ready for outplanting of the adults.

Figure 7. Proportion of *Bugula neritina* larvae swimming after 15 minutes and 1 hour 40 minutes of exposure to PW. The proportion of larvae attached at 1 hour and 40 minutes is also shown. Note the difference in scales.



By the time for outplanting the *B. neritina* colonies, there was no difference in the size of the colonies as measured by the number of zooids (not bifurcations), between the treatments (table 17). On average colonies were 5.5 ± 0.14 ($\times \pm$ SE) zooids in size. Despite a difference in the size of the batches of larvae, there was no interaction between treatment and batch.

Table 17. The size of *Bugula neritina* colonies at outplanting to the field.

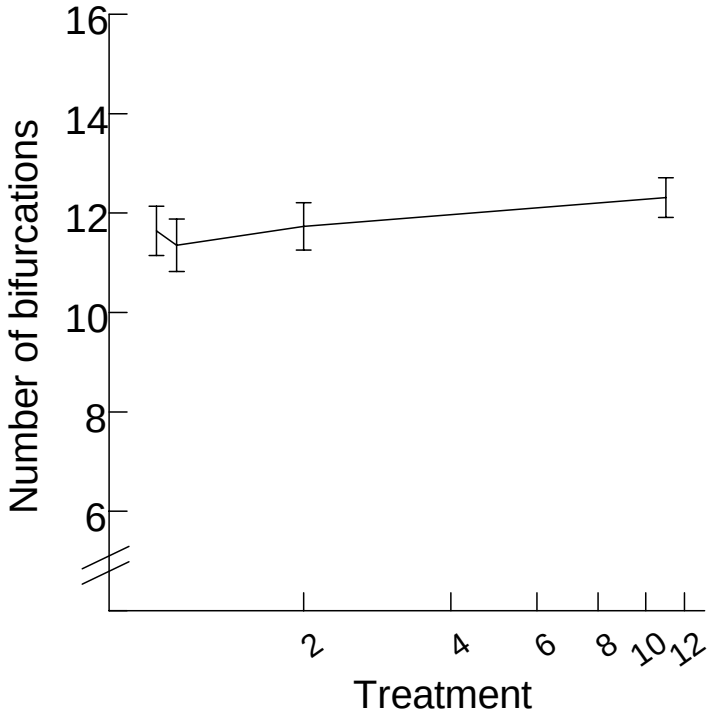
Source	**df**	**MS**	**F-ratio**	***p***
Batch	3	11.510	4.574	0.005
Tmt	3	0.280	0.111	0.953
Batch x Tmt	9	2.248	0.894	0.533
Error	119	2.516		

After 70 Days in the field, there was no difference in the size of the colonies as measured by the number of bifurcations (table 18, figure 8).

Table 18. The size of *Bugula neritina* colonies after 70 days in the field.

Source	**df**	**MS**	**F-ratio**	***p***
Tmt	3	2.634	0.790	0.505
Error	58	3.336		

Figure 8. The size of *Bugula neritina* colonies after 70 days in the field, measured by the number of bifurcations present on colonies.

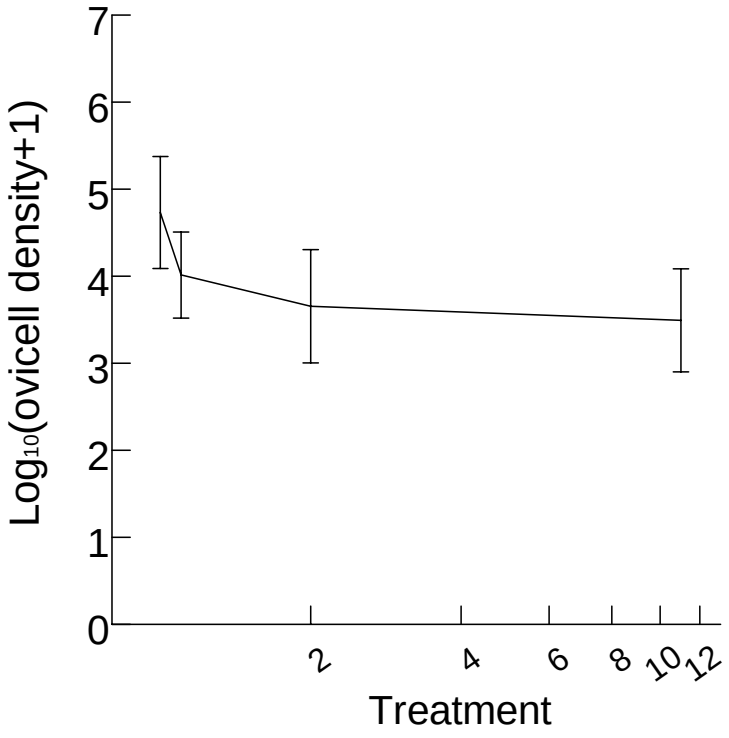


Interestingly there was no difference in the density of ovicells present on the colonies after 70 days in the field (table 19, figure 9). The number of ovicells were scaled for the size of colonies (number of bifurcations). Not surprisingly, there was a great deal of variability in the number and density of ovicells (note figure 9 is on a log scale). Despite this variability, there is a trend to decreased reproductive output as PW concentration increases. We counted all ovicells (full and empty).

Table 19. The size of *Bugula neritina* colonies after 70 days in the field.

Source	**df**	**MS**	**F-ratio**	***p***
Tmt	3	4.429	0.875	0.460
Error	**58**	**5.064**		

Figure 9. The density of ovicells present on *Bugula neritina* colonies after 70 days in the field, measured as Log_{10}(ovicell density+1).



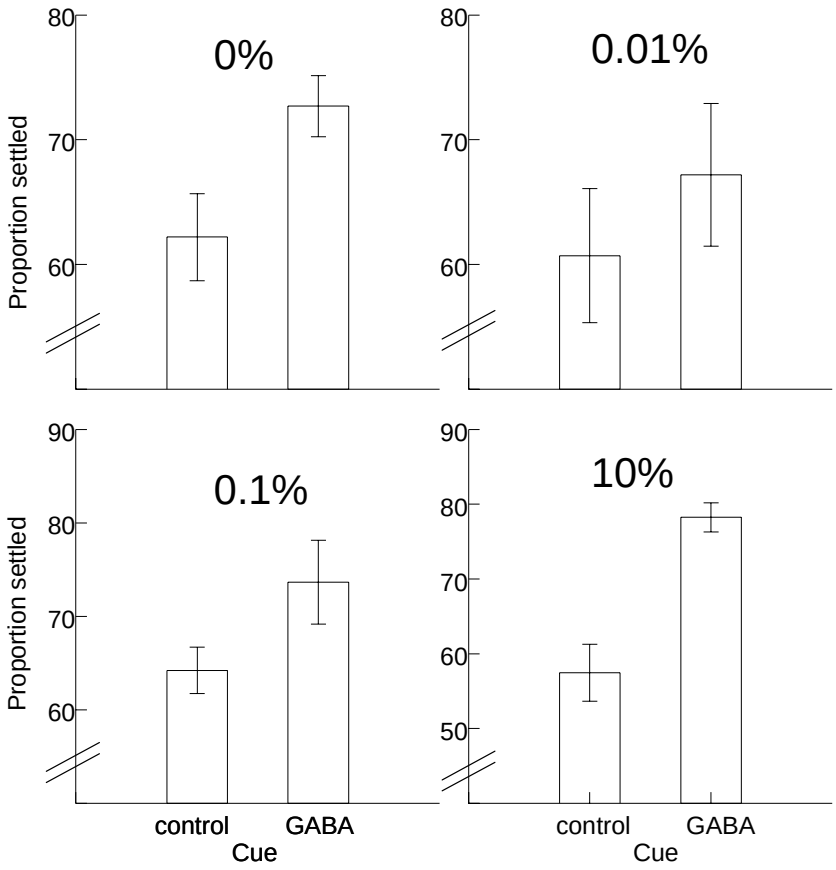
Haliotis rufescens

We did a small experiment using the larvae of *Haliotis rufescens* comparing the complex behavior of settlement cue recognition amongst larvae exposed to four levels of PW for one hour. Not surprisingly, proportionally more larvae settled when the cue (GABA) was used than in the control. After 32 hours had elapsed since exposure, there was no difference between the behavior of larvae in all PW treatments (table 20, figure 10).

Table 20. The proportional settlement of abalone larvae in four PW treatments (05, 0.01%, 0.1% and 10%) with and without a settlement cue. These data did not require transformation.

Source	**df**	**MS**	**F-ratio**	***p***
Cue	1	1070.249	27.314	0.000
PW	3	33.548	0.856	0.478
Cue*PW3	74.645	1.905	0.157	
Error	23	39.183		

Figure 10. The proportional settlement of abalone larvae in four PW treatments (05, 0.01%, 0.1% and 10%) with and without a settlement cue.



Discussion

Overall, this project found there was little evidence for strong sub-lethal effects that carried over from the larval phase and impacted the growth or competitive abilities of the subsequent *Watersipora subtorquata* adults (see also Boxshall and Raimondi, *in prep. A*). The studies of *W. subtorquata* were done three times across three different seasons. Each time, the results were the same. There is certainly evidence for (at times) quite strong sub-lethal impacts on the larvae of *Watersipora subtorquata* and the other larvae studied. Although not as extensively studied, the other invertebrates in this study showed similar patterns for the adults (see also Boxshall and Raimondi, *in prep. B and C).* Although this is an important finding for the protection of some planktonic organisms from impacts of the release of PW, there are a number of caveats.

One important caveat is that although mortality was generally low and not statistically significant, as would be expected from tests deigned to have deliberately sub-lethal impact, there were small differences seen in mortality of adults from larvae exposed to different concentrations of PW. When mortality occurred, it tended to be larger for *Watersipora subtorquata* colonies in treatments with spiked exposure to concentrations of and above 10% pre-release levels. These may be biologically interesting. Marine assemblages are highly variable, where there can be large variations in natural mortality from many sources (e.g., Connell 1978). Such small changes in adult mortality due to PW could simply be swamped by the natural high variability of marine populations. This caveat should be addressed by further work, possibly modelling of impacts (see Forde *et al.* in press).

Higher concentrations of PW affected larval behaviour of *Watersipora subtorquata* during exposure. Larvae generally swam less, settled less and showed less movement of any kind under the influence of higher concentrations of PW. This may be due to a narcotic effect from an unidentified constituent of PW. This speculation is based on the evidence that larvae placed into clean, filtered seawater immediately began to show a range of 'normal' behaviours. Generally there was little difference in the activity of the larvae within hours of exposure to PW, if they were washed in filtered seawater. Where there was larval mortality it tended to be in higher concentrations and was visible after the first hours of exposure. Although, as stated above, mortality in most of these experiments was low.

One important question that arises from these data is that if PW does not greatly impact the growth rates of invertebrates as adults, what is the fate, in a field situation, of those larvae that have had their behaviours altered? It is important to note that the all the larvae in this study were exposed in a laboratory situation. In this benign environment, individual larvae could be studied, followed, cleaned of PW and continue to live. If larvae pass through a cloud of PW in the ocean, stop swimming and sink, do they start to swim again once out of the PW? Or is there another fate? Are they more susceptible to predators? What about delayed metamorphosis and development? Does this leave them open to other natural impacts in the field? These remain unanswered questions.

Information from the outplanting of *Schizoporella unicornis* colonies after the exposure of larvae to PW was unfortunately truncated due to field logistics however there are some data being

reconstructed (Boxshall and Raimondi, *in prep. B*). At the time of outplanting there was little statistical difference in the size of *S. unicornis* colonies, although the pattern of increased size with increasing PW concentration was not continued through to the 10% PW concentration. Further study will be needed on the impact on subsequent adults to strengthen conclusions. There were very clear sub-lethal impacts on the larvae of *S. unicornis* that tracked increasing PW treatment quite well. The higher the concentration of PW, the less the swimming ability of newly released *S. unicornis* larvae. When these same larvae began to attach and metamorphose, the pattern of metamorphosis similarly tracked the PW concentration.

Like the other two bryozoans, there were strong sub-lethal impacts of PW on the behavior of larvae of *Bugula neritina*. Also like the other bryozoans, the carry-over impacts from the larval phase to adulthood were not strong. In the case of *Bugula neritina*, we were able to track the adults for 70 days in the field and follow their growth as well as gain some insight into their reproductive capacity. It is important to note that this measure of reproductive output can only be viewed as a single, snap-shot impression of the reproductive output from *Bugula neritina*. If it would be possible logistically, a better estimate of reproductive output would be to count the life-time output from adult colonies. From our single estimate of reproduction, there was no strong impact carried-over from larval exposure to different concentrations of PW. This result should be viewed as a precursor to more analysis. Very late in the period of this project, access was granted to new supplies of PW by MMS. With this access, it was possible to re-visit the question of carry-over impacts on reproductive success of *B. neritina*. As a consequence, more data was generated after the project time had concluded. This is being further analyzed and will be published in future (i.e., Boxshall and Raimondi, *in prep. B*).

Given the sub-lethal impacts previously seen in field studies using the abalone *Haliotis rufescens* (Raimondi and Schmitt 1993) it was surprising to note that the complex behavior of cue recognition by the abalone was not impacted by PW exposure. The swimming capacity of *Haliotis rufescens* larvae were impacted during other pilot tests (Boxshall, unpubl. data).

On the topic of PW concentrations, as previously discussed, exposure to 10% PW concentrations for one hour is unrealistically high exposure based on current plume dilution studies. This concentration was deliberately chosen to elicit a behavioural response (which it did) and to allow us to follow these larvae/adults through life to look for long-term effects. The 25% PW was used for similar reasons but no adults of any species were tracked through life. One important source of error in this project was the origin of the PW stocks. As noted, PW used for experiments came from 2 collections on single days at single (unknown) platforms. If there is to be the capacity to generalise about the impacts of PW (*per se*) on the ecology of marine organisms, studies must be done using a range of PW from a range of sources. The composition of PW is known to be very variable (see multiple papers in both Ray and Engelhardt, 1993 and Schuurmann and Market, 1997).

There were very few interactions with treatment produced by exposing the larvae to PW in batches or as one large pool. When larvae were not batched, except for this 50 - 65 minute period, the larvae and subsequent adults were raised and monitored individually for the duration of the experiment. Of course, the exposure is an important time. The practice of batching larvae

is in sharp contrast to common procedure in many LC50-96 tests where organisms are treated and monitored as pooled groups throughout the study. The behaviors of larvae are inherently variable (see any paper in McEdwards, 1995) and so this issue should not be dismissed. However, it should also be seen within the context of the previous discussion about the lack of replication in sources of PW.

When effects from batching larval exposure were seen, they tended to occur in later in the early stages of young adult development, not in the early larval swimming, metamorphosis or survival in the first 24 hours. Batch effects were also generally not present later as adults (e.g., growth at outplanting, and both growth and survival later). However, there were batch effects in the development of opercula of the bryozoan *W. subtorquata* (Day 4) in the laboratory and the survival of juvenile *W. subtorquata* to outplanting. These are early stages of development, but after metamorphosis. This is an interesting pattern worth noting but for which we have no present speculative solutions.

The competitive ability of *W. subtorquata* adults were assessed after 150 days in the field. This time was required for enough competitors to grow around the colonies. The overall competitive load was no different amongst colonies in different PW treatments. The competitors were mainly ascidians. No PW treatment showed a diminished ability to compete. The unfortunately low power in these tests is difficult to overcome due to the variable and unpredictable nature of settlement in the field. The design of this experiment is ultimately controlled by where and when competitors settle.

This project was successful in addressing the question of carry-over impacts from exposed invertebrate larvae to the adult phase of their life-cycle. A number of important further questions were raised as a result of this study. During this project, various personnel were involved, gaining valuable learning experience. They are listed in Appendix 1.

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The Department of the Interior Mission

As the Nation's principal conservation agency, the Department of the Interior has responsibility for most of our nationally owned public lands and natural resources. This includes fostering sound use of our land and water resources; protecting our fish, wildlife, and biological diversity; preserving the environmental and cultural values of our national parks and historical places; and providing for the enjoyment of life through outdoor recreation. The Department assesses our energy and mineral resources and works to ensure that their development is in the best interests of all our people by encouraging stewardship and citizen participation in their care. The Department also has a major responsibility for American Indian reservation communities and for people who live in island territories under U.S. administration.

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As a bureau of the Department of the Interior, the Minerals Management Service's (MMS) primary responsibilities are to manage the mineral resources located on the Nation's Outer Continental Shelf (OCS), collect revenue from the Federal OCS and onshore Federal and Indian lands, and distribute those revenues.

Moreover, in working to meet its responsibilities, the **Offshore Minerals Management Program** administers the OCS competitive leasing program and oversees the safe and environmentally sound exploration and production of our Nation's offshore natural gas, oil and other mineral resources. The MMS **Royalty Management Program** meets its responsibilities by ensuring the efficient, timely and accurate collection and disbursement of revenue from mineral leasing and production due to Indian tribes and allottees, States and the U.S. Treasury.

The MMS strives to fulfill its responsibilities through the general guiding principles of: (1) being responsive to the public's concerns and interests by maintaining a dialogue with all potentially affected parties and (2) carrying out its programs with an emphasis on working to enhance the quality of life for all Americans by lending MMS assistance and expertise to economic development and environmental protection.

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ACKNOWLEDGEMENTS

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EDC: A HISTORY OF ADVOCACY ON OFFSHORE OIL

Founded in 1977 in response to the 1969 Santa Barbara oil spill, the Environmental Defense Center fills a critical gap as the only non-profit environmental law firm between Los Angeles and San Francisco. Serving Ventura, Santa Barbara, and San Luis Obispo Counties, EDC provides public education, advocacy, and legal services to non-profit organizations dedicated to environmental quality and human health.

Protecting our coastal environment and communities from the risks and impacts of offshore oil development has been integral to EDC's work since our founding. In 1999, EDC led a successful statewide legal fight against federal offshore oil leases, preventing the extension of 36 leases for offshore oil production.[1] The leases had been issued between 1968 and 1984, but had never been developed. Representing a broad coalition of environmental organizations, EDC joined with the California Coastal Commission to file a lawsuit challenging the extension on the grounds that the federal Minerals Management Service had failed to let the Coastal Commission conduct a consistency review pursuant to the Coastal Zone Management Act, and had failed to conduct environmental review under the National Environmental Policy Act.[2]

The case was decided in EDC's favor at both the district court and appellate level. EDC's success was not only significant to the local community, but also established an important legal precedent by giving coastal states greater authority to review and prevent federal actions that could impact their communities and environments.

EDC's work has also been integral in efforts to improve regulatory oversight of air pollution and wastewater discharges from platforms located in federal waters.[3] Through our representation of dozens of groups fighting offshore oil drilling, EDC's work has helped stop further oil development, prevent oil spills, protect threatened and endangered species, and reduce air and water pollution. In addition to protecting our local environment, EDC's offshore advocacy efforts have been motivated by a desire to reduce greenhouse gas emissions and to encourage the move away from fossil fuels and towards renewable energy sources.

EXECUTIVE SUMMARY

In DIRTY WATER: FRACKING OFFSHORE CALIFORNIA, the Environmental Defense Center (EDC) addresses the oil industry's use of hydraulic fracturing (*aka* fracking) and other forms of well stimulation from offshore platforms located within federal, Outer Continental Shelf (OCS) waters in the Santa Barbara Channel. The Santa Barbara Channel and the Channel Islands are renowned globally for their beauty, richness of wildlife, and overall health of the environment. Although fracking has been conducted off of California's shores for at least two decades, the practice was until recently largely unknown to state and federal regulators, as well as the general public.

Fracking has been conducted from platforms off California's coast for 20 years, but until this year was largely unknown to state and federal regulators and the public. © Linda Krop.

EDC's review and analysis of federal records received through the Freedom of Information Act (FOIA) show that at least 15 fracs have occurred offshore California, with several more proposals pending. More fracs have almost certainly been conducted, however, as federal regulators were until recently unaware that the practice was being used. The information currently available shows that the majority of fracs have occurred from platforms with a history of spills that are in close proximity to the Channel Islands National Marine Sanctuary and other ecologically important areas.

The revelation that fracking is occurring off California's shores comes three years after the largest offshore oil spill in our nation's history, the 2010 BP Deepwater Horizon disaster. **Like the 1969 Santa Barbara oil spill, Deepwater Horizon occurred after federal regulators had granted the industry waivers or shortcuts from environmental and safety requirements. In its wake, the Obama administration claimed to launch the largest reform of offshore oil oversight in the nation's history.**

Important aspects of the administration's effort, however, including reform of the Department of the Interior's (DOI) environmental analysis of OCS proposals under the National Environmental Policy Act (NEPA), remain unfulfilled. DOI's oversight (or lack thereof) of offshore fracking in the Santa Barbara Channel illustrates this lack of reform, and also raises questions of compliance with other major environmental laws including the Clean Water Act and Coastal Zone Management Act.

In order to guard against an offshore drilling disaster involving fracking off California's shores, EDC recommends that the Obama administration:

- Place a moratorium on offshore fracking and other forms of well stimulation unless and until such technologies are proven safe through a public and transparent comprehensive scientific review
- Prohibit the use of categorical exclusions (exemptions from environmental review) to authorize offshore fracking and other forms of well stimulation
- Formally evaluate offshore fracking and other forms of well stimulation through a Programmatic Environmental Impact Statement
- Initiate consistency reviews with the California Coastal Commission for all exploration plans, development plans, drilling or modification proposals involving fracking and other forms of well stimulation
- Ensure that all fracking proposals comply with the Endangered Species Act and Marine Mammal Protection Act
- Review and revise the Clean Water Act permit for offshore platforms to specifically address fracking and other forms of well stimulation

Both the devastating 1969 Santa Barbara oil spill and the 2010 BP Deepwater Horizon blowout occurred after federal regulators granted industry shortcuts from environmental safeguards. © Robert Sollen

With the 1969 oil spill, California's south central coast experienced the devastating impact of one of the largest environmental disasters in U.S. history. These communities learned first-hand what can happen when government agencies turn a blind-eye to industry practices. This year, with all levels of government awakening to the existing reality of fracking off our precious coastline, this is not the time to repeat the mistakes of the past, but rather to focus on solutions to help avoid the worst impacts of the accidents that are all but inevitable and all too often realized. This report has been designed to outline some of these essential solutions.

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INTRODUCTION

The Environmental Defense Center (EDC) analysis of federal government records received through the Freedom of Information Act (FOIA)[4] reveals that the oil industry has been utilizing hydraulic fracturing (*aka* fracking) and related well stimulation techniques to increase oil production from oil platforms located off California's coastline for at least twenty years. The use of fracking off California's shores was largely unknown to federal and state regulators, as well as the general public, until two teams of investigative journalists reported on the issue in summer 2013. [5]

California's south central coast has long lived with the threats posed by offshore drilling. The realization that unregulated fracking is also taking place should serve as a wake-up call to ensure the protection of our environment and local communities. © Linda Krop.

Fracking involves pumping a mixture of water, sand (known as "proppant"), and chemicals down a well at extremely high pressures to break apart a hydrocarbon-bearing geologic formation and improve rates of oil or natural gas production.[6] Although rudimentary forms of fracking have existed for decades, today's technology is the first to successfully produce large quantities of oil and gas from the dense sedimentary rock known as shale.[7]

These advancements, together with other improvements in horizontal drilling technologies allowing access to larger areas of the formation, have served to vastly increase shale oil and gas production during the past decade.[8] In 2000, shale gas comprised 1 percent of domestic supplies; today, that figure exceeds 35 percent and is expected to grow further.[9] According to industry, nine of ten oil and gas wells today require some form of fracture stimulation in order to be economically viable.[10]

> *"Oil and gas development, whether conventional or shale oil and gas, poses inherent environmental and public health risks, but the extent of those risks associated with shale oil and gas development is unknown."*
>
> *- U.S. Government Accountability Office*[18]

The Energy Information Administration (EIA) recently identified California's Monterey Shale, encompassing large portions of the southern and central portions of the state, both on and offshore, as the nation's largest oil shale "play."[11] The agency estimates that the Monterey harbors 15.4 billion barrels of "technically recoverable" oil, more than 60 percent of the nation's total estimated shale oil resources.[12] Although a true oil shale boom has not yet occurred, **fracking for shale in California is already on the rise, with at least 1,200 fracs performed in the state since January 2011**.[13]

The technological advancements driving today's "modern" fracking pose new and largely unstudied environmental and public health risks that are cumulative to the significant impacts arising from "traditional" oil and gas production.[14] For example, today's fracking relies on "frac fluids" containing extensive amounts of chemicals, many undisclosed under trade secret and other business confidentiality laws.[15] Compared to past practices, fracking is now conducted further below the surface (often more than two miles), down wells that pass through groundwater aquifers commonly relied upon for domestic and agricultural use.[16] And fracking today relies on the use of much larger quantities of increasingly scarce freshwater supplies than past oil and gas operations.[17]

Conducting modern fracking techniques offshore adds yet another layer of complexity, uncertainty and risk. As one top federal regulator said during a recent spill, offshore drilling is "inherently risky" and blowouts "aren't that infrequent."[19] In light of this inherent risk, offshore fracking is obviously of great concern. While limited information is available, most offshore California fracs to date appear to be what are known as a "frac pack," a modified version of a "gravel pack." Both methods are intended to create a sand filter that serves to control sand production in poorly bonded offshore formations.[20]

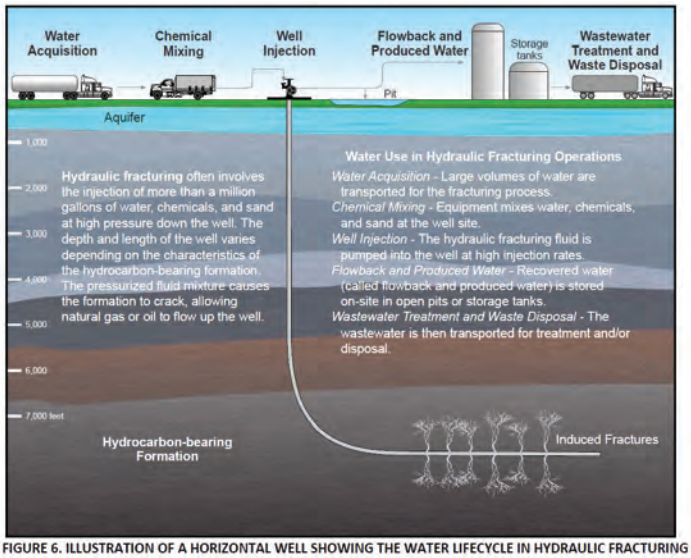


FIGURE 6. ILLUSTRATION OF A HORIZONTAL WELL SHOWING THE WATER LIFECYCLE IN HYDRAULIC FRACTURING

All across California and throughout the United States, communities have been facing an onslaught of new onshore fracking operations, however almost no one realized that secretly oil companies have been fracking in our fragile ocean for at least two decades. Image from EPA Hydraulic Fracturing Study Plan.

The past use of offshore "gravel packs" did not, however, involve fracturing the formation.[21] While the "frac pack" technique differs in some respects from onshore fracking (for example, using larger quantities of sand and using seawater in place of fresh water), the core process is the same: the injection of water, sand, and chemicals at high pressures with the intent of exceeding the fracture pressure of the geologic formation,[22] but doing so under the seabed.

This report, focusing on federal, "outer continental shelf" (OCS) waters, located beyond three nautical miles from the state's coast, explores the natural resources that are at risk from offshore fracking, the known frequency and extent of the practice off California's shores, recent lessons that may be drawn from the Deepwater Horizon disaster, and questions of compliance with several federal environmental laws. It ends with a series of recommendations for addressing the newly discovered practice of offshore fracking with a focus on the Santa Barbara Channel.

California on Acid

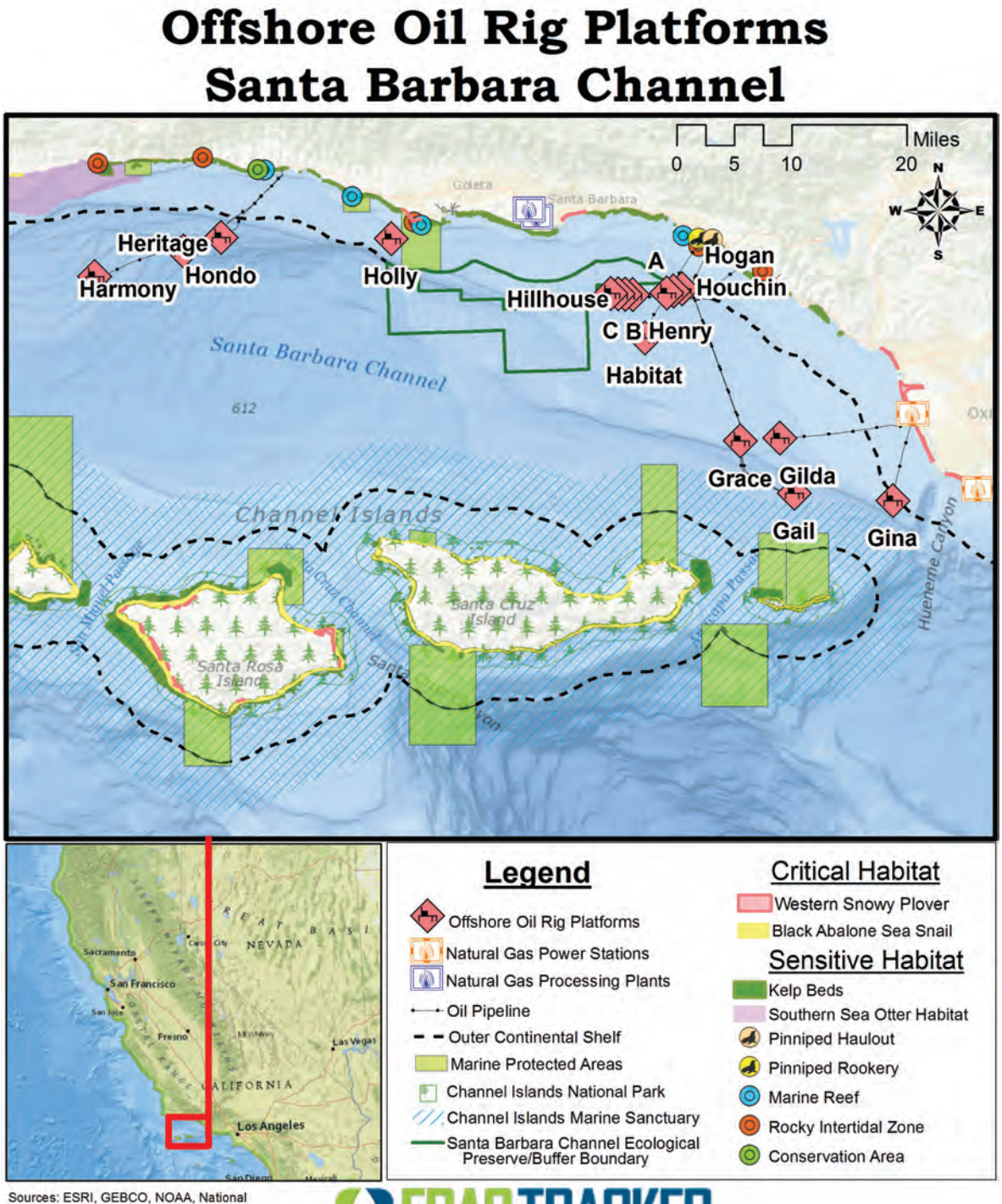
Despite the vast estimates of oil reserves harbored in the Monterey Shale, it remains unclear whether fracking can "unlock" those resources.[23] While shale formations in other areas of the country commonly trap oil in flat layers, seismic forces have folded the Monterey Shale formation.[24] Because of this geologic complexity, many oil industry insiders believe the formation may respond better to alternative stimulation techniques, such as "acidizing," that open small pores in the rock, than to hydraulic fracturing.[25]

As the name implies, acidizing involves the use of hydrofluoric and hydrochloric acids, some of the most hazardous industrial chemicals in use, to stimulate well production.[26] Like fracking, rudimentary forms of acidizing have been used for decades, but are now being utilized in new and more intensive ways. According to the state Division of Oil, Gas & Geothermal Resources (DOGGR), the two primary forms of the process used in California are "fracture acidizing" and "matrix acidizing."[27] Fracture acidizing is "similar to [fracking] in that pressures are done at the fracture gradient of the hydrocarbon bearing formation to create the fractures," but "differs in that proppants are not used."[28] Matrix acidizing is "similar to fracture acidizing except it is performed below fracture pressure and is used to dissolve channels to create wormholes near the wellbore."[29]

Available information, though limited, indicates that acidizing has been commonly utilized on offshore platforms within the Santa Barbara Channel.[30] The precise extent and frequency of fracking, acidizing, and other well stimulation methods in California has not been transparent to the public, as the practice has not been specifically regulated or tracked by federal or state regulators. Although California Governor Jerry Brown signed Senate Bill 4 on September 20, 2013, a lengthy and complex piece of legislation that, among many other provisions, establishes a permitting system for fracking and acidizing proposals [31], this legislative mandate applies to proposals onshore California and in offshore state waters, and not to federal waters that are the focus of this report.

WHAT'S AT STAKE

The large majority of California's federal offshore oil platforms are located within the Santa Barbara Channel, an arm of the Pacific Ocean separating Santa Barbara, Ventura, and other coastal communities from the northern Channel Islands. Even in a state as renowned for its natural resources as California, the Channel stands out for its exceptional beauty and extraordinary biological diversity. Cool, subarctic waters converge with warmer, equatorial waters in the Channel, fostering a richness of marine and other wildlife, including blue, fin, humpback, minke, and killer whales, porpoises, dolphins, pinnipeds (seals and sea lions), the southern sea otter, and hundreds of species of birds, fishes, and invertebrates.[32]



At the outer boundaries of the Santa Barbara Channel, the Channel Islands harbor incredible biological diversity, so much so that they have been dubbed "North America's Galapagos." Reflecting the environmental importance of the area, the Channel Islands National Park (encompassing Santa Barbara, Anacapa, Santa Cruz, Santa Rosa, and San Miguel Islands) was established in 1980.[33] In total, the Islands and their surrounding waters provide habitat for more than 2,000 species of plants and animals, including 150 endemic species uniquely adapted to their island ecosystems and found nowhere else in the world.[34]

Also established in 1980, the Channel Islands National Marine Sanctuary (CINMS) encompasses 1,470 square miles of ocean habitat around the islands.[35] The CINMS is one of only 14 such marine sanctuaries nationwide, established under federal legislation for their conservation, recreational, ecological, historical, scientific, cultural, archeological, educational, or aesthetic qualities.[36] Notable species that take refuge in the Sanctuary include over 25 species of whales and dolphins, five species of seals and sea lions, more than 20 species of sharks, and over 60 species of birds.[37]

Santa Barbara Channel has been called North America's Galapagos due to richness of habitat and over 2,000 species of plants and animals. © Linda Krop.

More recently, in separate but related actions in 2002 and 2007, the State of California and the federal government established a network of marine reserves within the CINMS.[38] In 2012, California completed the United States' first statewide network of Marine Protected Areas (MPAs) along the California coast, designed to ensure healthy and vibrant populations of fish and other marine species.[39] Off the coast of Santa Barbara, these underwater preserves and parks can be found at Point Conception, Kashtayit (near Gaviota State Park), Naples Reef, Campus Point, the Goleta Slough, and several designated areas surrounding the Channel Islands.[40]

Santa Barbara County's Energy Division map features the 23 existing offshore platforms, including Platform A which is still in service 44 years after being the source of the Santa Barbara oil spill.

Despite its beauty and environmental importance, the Santa Barbara Channel has long been the epicenter of California offshore oil development, both physically and symbolically. The nation's first offshore wells were drilled along this coast, and it was here that the nation's first large offshore oil disaster occurred. The 1969 Santa Barbara oil spill, which still looms large in the public's consciousness, is widely credited with catalyzing enactment of landmark state and federal environmental legislation. The spill, however, failed to substantially slow federal leasing and permitting decisions that opened up California's waters to extensive offshore oil development. Between 1967 and 1984, the U.S. Department of the Interior (DOI) sold 311 leases covering more than 1.6 million acres off the California coast.[41] Today, 23 offshore platforms still operate in the Santa Barbara Channel, all but one of which is located in federal, rather than state waters.[42]

The 1969 Santa Barbara Oil Spill

On the morning of January 28, 1969, while the Union Oil crew on Platform A was retrieving pipe from the bottom of a well drilled five miles offshore Summerland, California, something went terribly wrong: the well blew out.[43] As oil began seeping up from the bottom of the ocean floor, so began one of the largest environmental disasters in U.S. history.[44]

Oil saturated the Santa Barbara Channel and washed ashore for eleven days before the well at Platform A was capped—yet even after the well was capped, oil continued to seep up steadily through fractures in the ocean floor for several years.[45] In the end, over three million gallons of oil were released, fouling 35 miles of coastline, killing as many as 15,000 seabirds, and poisoning dolphins, seals and sea lions.[46] Santa Barbara's tourist-dependent local economy, businesses and property owners, and the local fishing industry all suffered extensive economic loss in the wake of the spill.[47]

The 1969 oil spill gave birth to the environmental movement and a slate of laws to help protect community and environmental health, and yet two generations later Californians have learned that oil companies are conducting risky offshore fracking operations without adequate regulatory oversight. © Robert Sollen.

Prior to the spill, industry had claimed that safeguards were in place to prevent such a blowout.[48] So what happened? The cause of the blowout has been primarily attributed to Union Oil's use of an improper casing.[49] Casing is used to reinforce a well and thereby prevent blowouts. On Platform A, federal regulators provided Union Oil with permission to use a shorter casing than normally required by federal standards.[50] Casing the well at a shallower depth left the well unprepared to handle the pressure of the ensuing blowout.[51] In other words, the 1969 oil spill "might have been avoided but for a failure of federal oversight."[52]

The 1969 Santa Barbara spill is widely recognized as a catalyst for the enactment of many of the nation's most bedrock and enduring environmental laws, including the National Environmental Policy Act (NEPA)[53], Clean Water Act (CWA)[54] , and creation of the U.S. Environmental Protection Agency (US EPA).[55] At the state level, Californians overwhelmingly passed a citizens' initiative (Proposition 20) in 1972, which lead to the passage of the Coastal Act in 1976, one of the nation's strongest environmental laws.[56]

FRACKING OFF CALIFORNIA'S SHORES
PRELIMINARY INFORMATION BUT AN INCOMPLETE PICTURE

In March 2013, EDC submitted a FOIA request to the Bureau of Safety and Environmental Enforcement (BSEE), an agency within DOI, in order to investigate whether there had been any instances of fracking from offshore platforms located in federal waters off the California coast. BSEE is responsible for permitting offshore drilling operations and ensuring that such operations comply with required safety regulations, while its partner DOI agency, Bureau of Ocean Energy Management (BOEM), conducts OCS lease sales and is responsible for environmental analysis under the Outer Continental Shelf Lands Act (OCSLA)[57], NEPA, and other laws.[58]

EDC's analysis of that FOIA response determined that at least 15 instances of fracking off California shores within federal waters have occurred over the last twenty years—with at least four frac jobs approved as recently as this year.[59]

Importantly, however, it is almost certain that the FOIA response does not accurately reflect the true frequency and extent of offshore fracking, as officials at BSEE appear to have been unaware that fracking was occurring until very recently. Indeed, BSEE did not begin to familiarize itself with the issue until prompted by questions from concerned citizens and the need to respond to various FOIA requests.[60]

Once it finally became aware of the practice, BSEE's estimates of the extent of fracking in the Santa Barbara Channel steadily grew over the course of several months, as reflected in the evolving agency drafts of a public "fact sheet," in which the agency revised its frac estimates from 2 to 4 to 11 to "very few" between January and April 2013.[61] (See Table 1). The fact sheet similarly shows evolving knowledge in relation to the type of fracking method being utilized, with agency staff incorrectly stating that horizontal fracking had not been utilized offshore (in fact, Venoco fracked horizontally off Platform Gail in 2010).

TABLE 1: EVOLVING BSEE FRAC ESTIMATES

January 11, 2013	2
February 11, 2013	4
February 25, 2013	11
April 2013	'very few'

Nor does it appear likely that DOI will be able to determine the true extent of California offshore fracking anytime soon, as its files are apparently not easily searchable.[62] As a BSEE spokesperson recently stated, "it cannot be sure just how often fracking has been allowed without going through every single well file."[63] In the Gulf of Mexico, BSEE estimates that 12 percent of offshore wells have been fracked.[64]

> *"To get the full number of fracs performed offshore, BSEE officials would have to comb through every well file and count the number of fracking operations, which could take years because many files are not digitized."*[65]

The records that have been located thus far by BSEE primarily document fracking from Platforms Gilda and Gail, both located in the "Santa Clara Unit" off the Ventura County coast.

Venoco was issued 32 violations at Platform Gail for not following basic operating procedures between 2005 and 2010. © Erin Feinblatt

Installed in 1987, Platform Gail is the closest of all Santa Barbara Channel platforms to the Channel Islands National Marine Sanctuary, located just outside its boundaries. It is also located in close proximity to Anacapa Island within Channel Islands National Park, and the Marine Reserve Area extending off that Island's northern shores.

Platform Gail, currently operated by Venoco, Inc., has a history of spills.[66] These spills have been caused by a variety of factors, including losses in well control[67] and pipeline ruptures.[68] A recent investigation found that **"Venoco was issued 32 violations … for not following basic operating procedures" in the years 2005–2010.[69] Unsettlingly, this was apparently "the smallest number of violations of any company working in the channel."**[70]

Table 2: Currently Known Offshore Fracks in Federal and State Waters

Platform	Location	Operator	Date
Esther	Offshore Seal Beach	DCOR	Unknown (State Waters)
Eva	Offshore Huntington Beach	DCOR	Unknown (State Waters)
Gail	SB Channel	Venoco	1992, 2010
Gilda	SB Channel	DCOR/Nuevo/Torch	1994, 1997, 1998, 2001, 2002, 2003, 2013
Hidalgo	Point Arguello Field	Chevron (now PXP)	1997

Platform Gilda was installed in 1981, and is currently operated by Dos Cuadras Offshore Resources, LLC (DCOR).[71] In recent years, DCOR has become the largest California offshore operator, managing eleven of the 23 producing platforms off the state's shores, including Platforms A (site of '69 oil spill), B, C, Gina and Henry.[72]

> *"Our position is that [offshore fracking is] safe and effective. It's just like they're out there in Kansas, except there's an ocean on top."*[77]
> *- International Association of Drilling Contractors*

Over the course of three weeks between the months of March and April in 2013, DCOR reported at least one spill per week from Platform Gilda.[73] Nonetheless, in May 2013, DCOR removed existing safety infrastructure from Gilda, including a spill boom deployment boat, and will instead rely on Oil Spill Response Vessels operated by Clean Seas as its primary response mechanism in the event of a spill.[74] DCOR has also been responsible for notable spills at other offshore platforms, including a leak of more than 1,100 gallons of oil from Platform A into the ocean due to a hole in one of the oil pump lines.[75] The resulting sheen reached 1.5 miles in length.[76]

From the information that EDC has been able to gain from our investigation, Platform Gilda appears to be the platform from which fracking most regularly happens, and yet in 2013 safety infrastructure was removed from the platform. © Erin Feinblatt

ACCIDENTS HAPPEN
ESPECIALLY WHEN THE GOVERNMENT GIVES SHORTCUTS TO THE OIL INDUSTRY

Taking note of the spills and other accidents at Platforms Gail, Gilda and the other platforms operated by Venoco and DCOR, draws attention to the fact that accidents happen. No process is perfect—and because no process is perfect, industry and government need to be vigilant when it comes to regulating offshore drilling and production. Such vigilance is particularly important when the oil industry is utilizing new technologies or significantly modified forms of existing technologies.

BP's 2010 Deepwater Horizon blowout in the Gulf released an estimated 205 million gallons of oil. It was later discovered that before the disaster, BP had received exemptions from federal regulators. © Julie Dermansky.

Unfortunately, the federal government has instead time and again provided the oil industry with exemptions, shortcuts, and other loopholes for risky offshore drilling operations. For several decades, the most tragic consequence of this lack of vigilance was the 1969 Santa Barbara oil spill. That changed in April 2010, when a blowout at BP's Deepwater Horizon—an ultra-deepwater oil platform located in the Gulf of Mexico—killed eleven crewmembers and led to the "largest and most prolonged" offshore oil spill in our nation's history.[78] Over the course of three months, an estimated 205 million gallons of oil were released into the Gulf.[79]

The massive spill had a devastating impact on marine life, including dolphins, whales, seabirds, and sea turtles.[80] Being "one of the most productive sea food industries in the world," the Gulf economy also took a major hit.[81] Ongoing human health impacts continue, including extreme respiratory problems, eye and skin irritation, nausea, and central nervous system damage in local residents and response workers.[82]

As was the case with the 1969 Santa Barbara oil spill, government regulators had eased the rules at the Deepwater Horizon platform. Prior to the disaster, BP's exploration plans and drilling permits had been approved under a "categorical exclusion" from the public participation and environmental analysis requirements of NEPA, even though deepwater drilling is a relatively new and inherently risky practice.[83] In addition, it was later determined that DOI lacked the resources to establish meaningful safety regulations.[84] These deficiencies led the National Commission on the BP Deepwater Horizon Oil Spill and Offshore Drilling ("National Commission"), which was created by President Obama to study the cause of the spill, to conclude that "absent significant reform in both industry practices and government policies," an accident such as Deepwater Horizon "might well recur."[85]

> *"Efforts to expand regulatory oversight, tighten safety requirements, and provide funding to equip regulators with the resources, personnel, and training needed to be effective were either overtly resisted or not supported by industry, members of Congress, and several administrations. As a result, neither the regulations nor the regulators were asking the tough questions or requiring the demonstration of preparedness that could have avoided the disaster."*
>
> *–National Commission on Deepwater Horizon spill* [87]

SHORTCUTS WITH DISASTROUS CONSEQUENCES

1969 Santa Barbara Oil Spill

© Robert Sollen

Federal regulators approve casing of Platform A to 239 instead of the standard 880 feet [86]

2010 Deepwater Horizon Spill

© Julie Dermansky

Federal regulators approve drilling of BP's Macondo well in water 5,000 feet deep under a categorical exemption to the National Environmental Policy Act

FUNFINISHED NEPA REFORM IN THE WAKE OF DEEPWATER HORIZON

In the aftermath of the Deepwater Horizon disaster, the Council on Environmental Quality (CEQ) released a report on the NEPA procedures for environmental review by the Minerals Management Service (MMS), the DOI agency previously responsible for overseeing offshore oil development in federal waters.[88] CEQ is part of the Executive Office of the President and was established in the wake of the 1969 Santa Barbara oil spill as part of NEPA. CEQ's primary responsibliities include the coordination of federal environmental efforts across agencies and oversight over federal agency compliance with NEPA.

NEPA's two primary purposes are to ensure that public officials consider the environmental impacts of their decisions before they are made, and to ensure that the government decision-making process is transparent and open to public participation.[89] To that end, it requires that agencies prepare an environmental impact statement (EIS) or environmental assessment (EA) for proposed federal actions. In some circumstances, a proposal that falls within a category of actions previously determined not to have an individual or cumulatively significant effect on the environment can be exempted from analysis.[90] Such a "categorical exclusion" (CE), however, cannot apply if there are "extraordinary circumstances."[91] Under DOI regulations, these circumstances include, but are not limited to: highly uncertain effects, or that involve unique or unknown risks; significant impacts on ecologically significant areas; significant impacts on listed species; and actions with highly controversial environmental effects.[92]

In its review, CEQ found that MMS overwhelmingly issued CEs for oil and gas exploration plans and drilling proposals in the Gulf of Mexico—including risky deepwater operations—based on a concept known as "tiering."[93] Tiering involves reliance on a previous, "bigger picture" or programmatic EIS or EA in review of a subsequent, site-specific proposal, and is intended to increase the efficiency of NEPA compliance by minimizing redundant environmental analysis.[94]

Although tiering is a valid concept that can help increase the efficiency of environmental review, CEQ concluded that MMS had used it in a manner that "was not transparent . . .and has led to confusion and concern about whether environmental impacts were sufficiently evaluated and disclosed."[95] Based on its findings, CEQ offered recommended reforms to improve NEPA analysis of offshore oil decisions:

- **Tiering and Site-Specific Analysis:** "perform careful and comprehensive NEPA review," including "site-specific information where appropriate" [96]
- **Transparency, Public Accountability, and Sound Decision-Making:** "ensure that NEPA analyses fully inform and align with substantive decisions . . . and that those analyses will be fully available to the public;" and "ensure . . . robust analysis of reasonably foreseeable impacts, including . . .low probability catastrophic spills" [97]
- **Categorical Exclusions:** "review the use of categorical exclusions for Outer Continental Shelf oil and gas exploration and development in light of the increasing levels of complexity and risk" [98]
- **Changed Circumstances:** "consider supplementing existing NEPA practices, procedures, and analyses to reflect changed assumptions . . . specifically, conclusions may change about the likelihood, magnitude, and environmental impacts of a major spill in connection with OCS oil and gas drilling activities" [99]

On the same day that CEQ issued its report, BOEM's Director Michael Bromwich released a memorandum announcing that the agency would undertake a comprehensive review and evaluation of the agency's use of CEs, followed by a public notice of its intent to conduct a "broad review" of its use of categorical exclusions.[100/101] In the interim, BOEM was to "narrow its use of categorical exclusions," and Director Bromwich specifically identified the "proposed use of new or unusual technology" as a factor that would trigger more detailed environmental analysis. [102]

The recommendations and pledges made in the CEQ report and by Director Bromwich were laudable. In response, the Obama administration claimed to have "launched the most aggressive and comprehensive reforms to offshore oil and gas regulation and oversight in U.S. history."[104] Unfortunately, three years later, there has been no further action.[105] DOI is yet to publish even a draft set of recommendations arising from the review initiated by Director Bromwich.[106] At least with respect to pledges of NEPA reforms and associated CEQ recommendations, those promises and recommendations remain unfulfilled.

> *"We are building a more robust and aggressive independent oversight agency based on the development of new tools and enhanced legal and regulatory authorities, as well as on the more aggressive use of existing tools. These changes in our regulatory framework and approach will serve to hold offshore operators accountable and ensure that the industry and the country are fully prepared to deal with catastrophic blowouts and oil spills like the Deepwater Horizon."*[103]
>
> *–Former BOEM Director Michael Bromwich*

In response to Deepwater Horizon, President Obama "launched the most aggressive and comprehensive reforms to offshore oil and gas regulation and oversight in the U.S. history." Three years later, however, there has been no further action. © Laurie Bailey

FEDERAL OVERSIGHT OF OFFSHORE FRACKING
IN CONFLICT WITH ENVIRONMENTAL LAWS?

In the Santa Barbara Channel, DOI's oversight and regulation of fracking and other well stimulation techniques - or lack thereof - falls short of its pledged NEPA reform efforts, and also raises significant legal concerns under other cornerstone federal environmental laws including the CWA and Coastal Zone Management Act.[107]

These shortcomings are compounded by the numerous loopholes and exemptions provided to the oil and gas industry under federal law. The most notorious of these exemptions, the so-called "Halliburton amendment" included in the 2005 Energy Policy Act (2005 Act), specifically exempted fracking from the protections otherwise provided in the Safe Drinking Water Act.[108] The Halliburton amendment, and additional oil and gas loopholes from NEPA and the CWA included in the 2005 Act, arose from recommendations made by Vice President Cheney's industry-dominated "Energy Task Force." The 2005 provisions only further tear at a badly frayed safety net of federal environmental and public health laws governing well stimulation, and oil and gas production generally.[109]

NATIONAL ENVIRONMENTAL POLICY ACT

As detailed above, BSEE staff was unaware of offshore fracking prior to public inquiries regarding the practice. Consequently, the agency was also unsure whether the fracking operations had undergone environmental review under NEPA. As one staffer asked, "has fracking ever been considered in a five-year plan and been assessed in any NEPA document for the area in question?" Another suggested that fracking offshore "would be better left to a separate NEPA assessment" and that it "might even be better to deal with [offshore fracking] in a future programmatic document that covers the entire region."[110]

> *"Has there been an EIS to assess the environmental consequences of fracking on the OCS? How can we begin to review permit requests without that?"*
>
> *- BSEE staffer*

The suggestion is a valid one. In California's first major fracking litigation under NEPA, a federal court recently held that the Bureau of Land Management (BLM), an agency within DOI, violated the law in relation to lease sales on public lands in central California.[111] The court concluded that BLM failed to "adequately consider the development impact of [fracking] when used in combination with technologies such as horizontal drilling," and that BLM's "finding of no significant impact" was "erroneous as a matter of law." In response, the agency has initiated a comprehensive EIS analysis to study potential impacts of fracking **prior** to leasing.[112]

A recent California public lands court case determined that prior to fracking more significant environmental review is required. This case dealt with onshore fracking; the risks of offshore fracking are even less well understood. © Erin Feinblatt

As unstudied as the risks of onshore fracking are, offshore fracking is even less well understood. Despite its staffs' own internal questions, the lack of any prior NEPA analysis directly addressing the practice, and only months after first becoming aware that fracking was even being utilized in the Santa Barbara Channel, in June 2013 BSEE approved four new fracs from Platform Gilda under a CE.[113] The CEs were "tiered" to an OCS Plan of Development approved more than thirty years ago in 1980.[114]

CLEAN WATER ACT

Congress enacted the Clean Water Act (CWA) to "restore and maintain the chemical, physical, and biological integrity of the nation's waters."[116] The CWA prohibits the discharge of any pollutant into U.S. waters without a National Pollution Discharge Elimination System (NPDES) permit.[117] At a minimum, NPDES permits must include technology-based effluent limitations, any more stringent limitations necessary to meet water quality standards, and monitoring and reporting requirements.[118]

Approximately half the platforms in the Santa Barbara Channel discharge their polluted wastewater directly into the ocean. © Erin Feinblatt

In a typical onshore oil production operation in southern California, oil wastewater byproducts, including "produced water" and "frac flowback", are commonly injected back into underground reservoirs, and thus are subject to federal requirements other than the CWA. In contrast, approximately half the offshore platforms in the Santa Barbara Channel discharge their wastewater directly to the ocean (including Platform Gilda), while the other half inject the pollution underneath the seabed (including Platform Gail).[119]

Since 1984, discharges from the Santa Barbara Channel offshore platforms have been regulated under a "general" NPDES permit which limits the volume of various discharges, including drilling fluids, drill cuttings, and produced water.[120] "Produced water" is the most common waste byproduct in aging southern California oil fields that typically yield far more of it, often called "brine," than oil. The permit also places limits on the concentration of various pollutants that may be present in said discharges, and establishes monitoring and reporting requirements. In spring 2013, the EPA issued its more recent revision of the permit.[122]

The revised permit, however, failed to address fracking fluids and the host of chemicals found within, reflecting the fact that officials at both DOI and EPA were largely unaware that offshore fracking was being conducted. Although DOI has not promulgated rules requiring chemical disclosure from OCS wells, one recent report identified 2,500 "hydraulic fracturing products" in frac fluids,[123] 650 of which contained chemicals that are known human carcinogens, hazardous air pollutants, or have been otherwise identified as risks to human health.[124]

© Branden Aroyan

Despite the permit's lack of specificity, the lack of chemical disclosure, the numerous harmful chemicals that are known to occur in frac fluids, and the fact that agency staff were largely unaware that fracking was even occurring during the permit renewal process, EPA staff recently reached the questionable conclusion that fracking fluids are considered a "well completion fluid," regulated under the general NPDES permit effluent limitations, and thus may be discharged along with produced water under the newly-revised permit.[125]

Cooking the Climate

In addition to questions of compliance with federal laws, the prospect of widespread fracking in California raises serious concerns in relation to an issue not yet comprehensively addressed by federal law: climate change. While some have argued that natural gas fracking can help address greenhouse gas emission reduction by acting as a "bridge fuel" from coal dependence to renewable energy (though the high methane emissions at many gas production sites can negate much of these reductions), California fracking largely targets carbon-intensive oil - a bridge to nowhere. According to the California Air Resources Board, the extraction and transportation of oil from some state oil fields equals the carbon intensity of Canadian tar sands.[126] *Fracking California for oil is not only bad news for an already warming world, it would likely undermine the state's ability to meet its low carbon fuel standard.*

COASTAL ZONE MANAGEMENT ACT

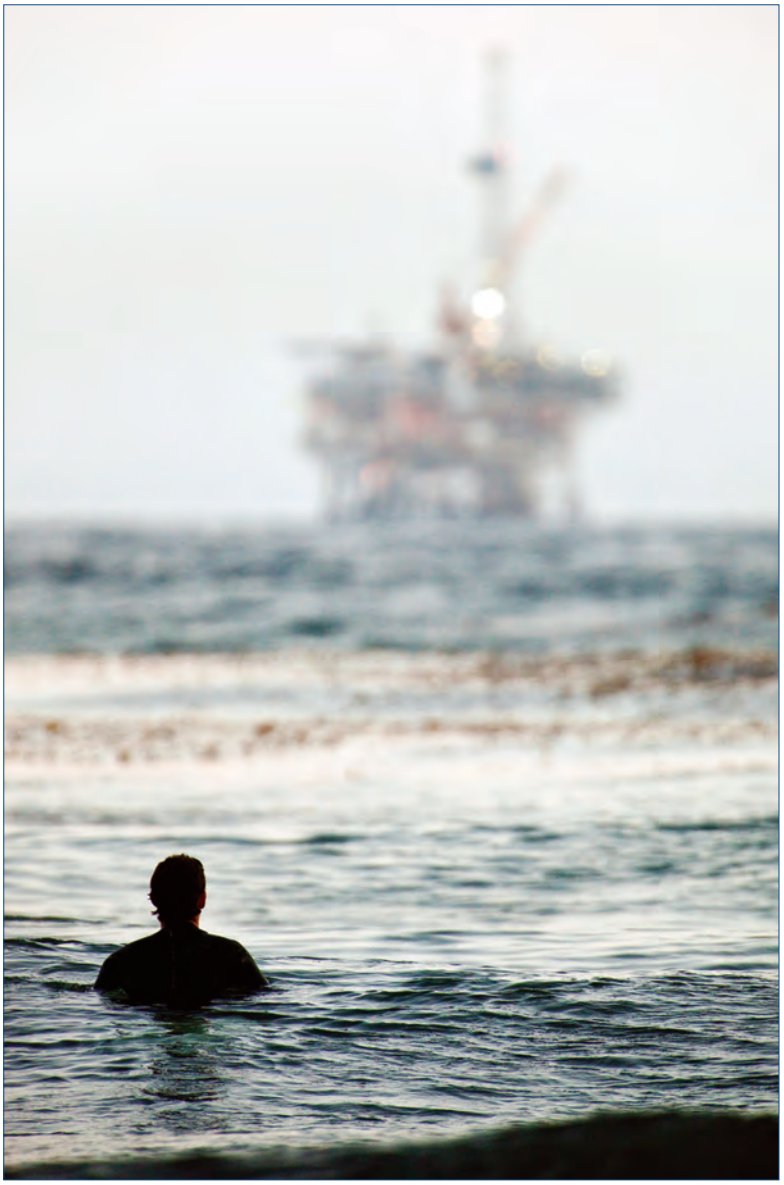
Congress passed the 1972 Coastal Zone Management Act (CZMA) in order to better define the respective jurisdiction of coastal states and the federal government in relation to coastal waters.[127] While the CZMA retained the existing three-mile federal boundary established by previous federal legislation, it also provided coastal states with oversight over activities in federal waters where those states have adopted a Coastal Management Program (CMP) to manage coastal land and water uses.[128] The CMP's purpose "is to encourage coastal states to manage their coastal resources in accordance with specific national priorities," including "protection of natural resources, water quality, shoreline stability, and public access."[129] In coastal states with federally approved CMPs, private entities that seek federal approvals such as permits or licenses must submit a "consistency certification" to the state showing that the activity is consistent with the CMP.[130]

Santa Barbara's 1969 spill from Platform A released over three million gallons of oil, fouling 35 miles of coastline, killing as many as 15 thousand seabirds and poisoning dolphins, seals, and sea lions. © Robert Sollen

"As President Nixon aptly observed, the Santa Barbara spill changed the nation's attitudes towards the environment. Some would trace the current framework of environmental protections in substantial measure directly to the Santa Barbara spill... Of particular relevance here, the federal Coastal Zone Management Act and California's Coastal Act followed in the wake of the spill and both provided California substantial oversight authority for offshore oil drilling in federally controlled areas." California v. Norton, 311 F.3d 1162, 1167 (9th Cir. 2002)

California's CMP, which goes well beyond the minimum protections mandated by the CZMA, is overseen by the California Coastal Commission, an agency generally regarded as rigorously protective of the state's unparalleled and irreplaceable coastal resources.[131] The Coastal Commission, as well as local California cities and counties, has a long history of conflict and disagreement with DOI concerning the federal government's offshore oil program.[132]

> *"We need to launch an investigation of offshore fracking done here in California. We do not yet understand the extent of fracking in federal and state waters, nor fully understand its risks."*
>
> *--Alison Dettmer, Deputy Director, California Coastal Commission*



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Under the CZMA consistency requirements, oil and gas companies seeking to conduct OCS exploration, development, or production must certify to DOI that the activity is consistent with the CMP.[133] Despite these requirements, California Coastal Commission staff in August 2013 stated that the agency "had no idea until recently that ocean fracking was even happening."[134]

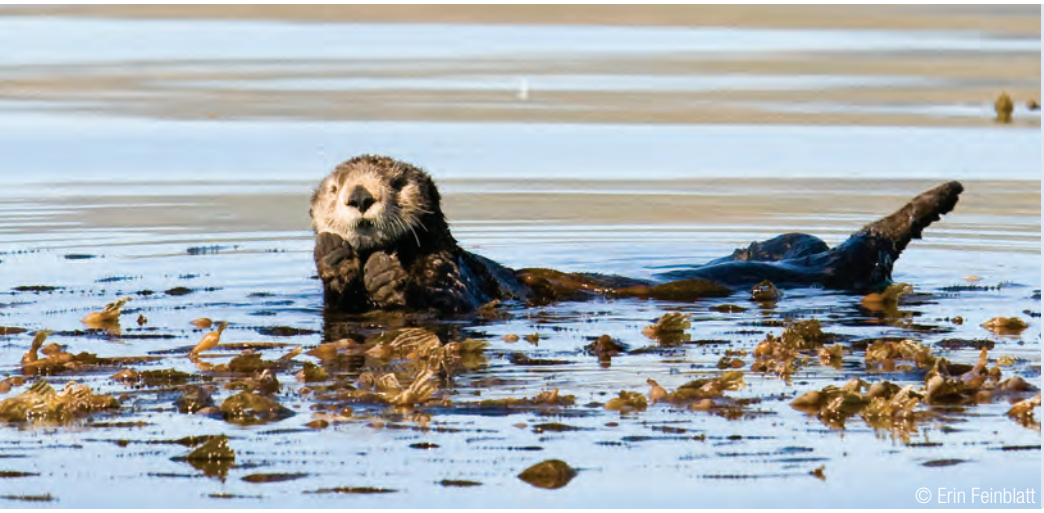
Although there are currently 23 platforms in offshore federal waters, the Commission has approved consistency determinations on the OCS plans for only 13 of these platforms—the rest predate establishment of the consistency review process by the state.[135] Compounding this gap in review, BSEE has been approving applications for permits to drill (APDs) and applications for permits to modify (APMs) as "minor revisions" to OCS plans. These plans have circumvented consistency review, as California's CMP only requires consistency reviews for "major revisions."[136]

However, given that BSEE itself was unaware of offshore fracking until recently, even if it had been conducting consistency certifications, those certifications would still not have included disclosure of fracking and analysis of its potential impacts. The Coastal Commission staff has launched its own investigation into the extent of offshore fracking, as well as the Commission's options under the CZMA consistency process and other authorities to address the practice.

> *"The coast is never saved. It's always being saved."*
>
> *–Peter Douglas, Founder of the California Coastal Commission and longtime Executive Director (1942-2012)*[137]

ENDANGERED SPECIES ACT AND MARINE MAMMAL PROTECTION ACT

Congress enacted the Endangered Species Act (ESA) to provide a means whereby endangered species and the ecosystems they depend upon may be protected.[138] The primary purpose of the ESA is not merely to prevent the extinction of listed species, however, but to recover them to the point where the protections of the Act are no longer necessary. To that end, the ESA's section 7 consultation provision requires that federal agencies ensure that actions they take or authorize do not jeopardize the continued existence of listed species, and requires them to consult with the Fish and Wildlife Service and/or National Marine Fisheries Service if protected species may be in the area and adversely affected by the proposed activity.[139]



Threatened and endangered species of the Santa Barbara Channel include the blue whale, fin whale, humpback whale, southern sea otter, black abalone, and white abalone.

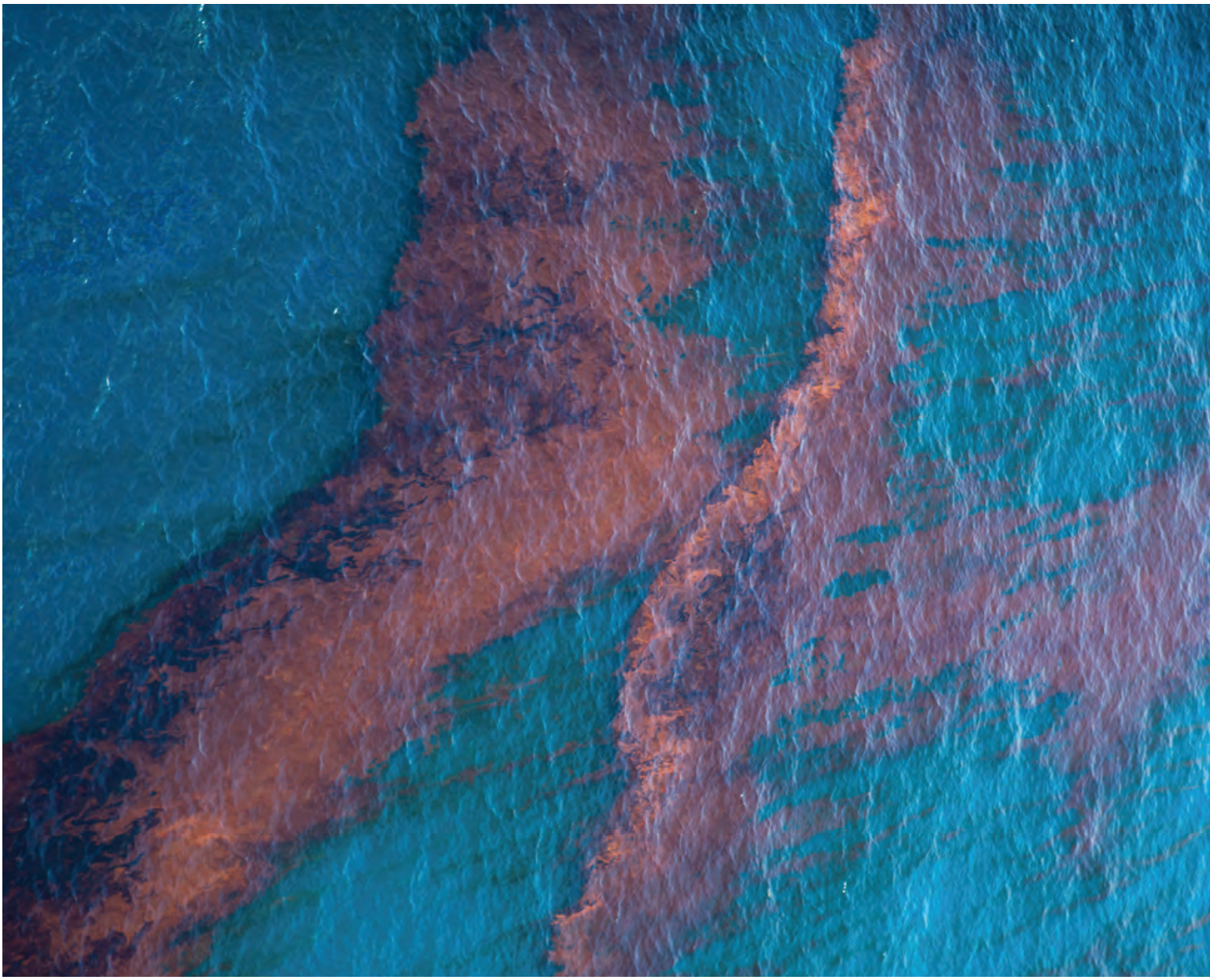
In addition, the Marine Mammal Protection Act (MMPA) provides overlapping but distinct protections to the marine mammals of the Santa Barbara Channel.[140] The MMPA provides additional constraints on federal agency actions, including a moratorium on "take" of marine mammals, defined as actions that cause disruption of migration, breathing, nursing, breeding, feeding, sheltering, or other essential behavioral patterns.[141] Actions that could incidentally take "small numbers" of marine mammals can be exempted so long as the activities are geographically limited and have a negligible impact, but such exemptions are only granted after a transparent public process.[142]

Unfortunately, there is no indication that DOI has considered the potential impacts of fracking on imperiled species of wildlife and other marine mammals in the Santa Barbara Channel. Even after learning that fracking is occurring, it appears that the agency will continue to approve future proposals with minimal environmental analysis, and without the benefit of complying with the mandates of the ESA and MMPA.

There is no indication that federal agencies are considering the impacts of fracking and polluted discharge on threatened and endangered species that live in the Santa Barbara Channel. ©Linda Krop.

RECOMMENDATIONS

If history is any guide, the federal government's lax oversight of fracking and other well stimulation practices within the Santa Barbara Channel is cause for significant concern. **Although the blame for the 1969 Santa Barbara oil spill and the Deepwater Horizon disaster ultimately lies with the oil industry, the likelihood of these disasters occurring could have been greatly reduced with robust federal oversight and aggressive implementation of laws and policies intended to protect the marine environment.**



The President's commission examining Deepwater Horizon concluded that without significant reform this type of disaster "might well recur." Unfortunately, there has been little movement in Washington. © Julie Dermansky

The National Commission concluded that "absent significant reform in both industry practices and government policies," an accident such as Deepwater Horizon "might well recur."[143] More than three years later, it is clear that such significant reform has not been achieved, particularly in the NEPA context, and appears to have been largely forgotten by the Obama administration. EDC has prepared this report in an effort to sound the alarm before yet another avoidable disaster occurs off our irreplaceable coastline. As this report focuses on offshore fracking within federal waters, our recommendations are accordingly focused on federal law and policy.

1. Moratorium on Fracking Until Further Environmental Review

DOI was largely unaware that fracking was occurring off California's shores until this year, and the agency is yet to consider and analyze the environmental risks of offshore fracking in a public and transparent manner. Accordingly, DOI should place a moratorium on fracking and other well-stimulation methods until it is able to assess the full extent of past, present, and potential future fracking off California's shores, and to thoroughly study the potential impacts of the technique on our coastal resources, water quality, extraordinary diversity of wildlife species, protected waters and lands, and critical economic drivers such as fishing and tourism. Further offshore fracking should only be conducted if it can be proven safe.

© Linda Krop

DOI may find some direction for its assessment under the independent study of fracking, acidization, and other well stimulation required by SB 4 for the State of California.[144] In this study, which must be completed by January 1, 2015, the California Secretary of Natural Resources must evaluate the hazards and risks well stimulation poses to "natural resources and public, occupational, and environmental health and safety," through a consideration of "at a minimum, atmospheric emissions, including potential greenhouse gas emissions, the potential degradation of air quality, potential impacts on wildlife . . . and habitat . . . induced seismicity, and the ultimate disposition, transport, transformation, and toxicology of well stimulation treatments."[145] As the study is targeted towards onshore fracking, DOI should develop a similarly comprehensive list of considerations tailored to the offshore marine environment.

2. Prohibit the Use of Categorical Exclusions For Offshore Fracking

DOI should establish enforceable policy prohibiting the use of categorical exclusions to authorize offshore fracking, acidization, and other well stimulation techniques in offshore waters. The use of CEs for offshore fracking is at odds with reforms proposed in response to Deepwater Horizon, precludes any environmental review or disclosure, and eliminates public transparency and participation. DOI must acknowledge that the use of fracking and other offshore well stimulation methods in the Santa Barbara Channel triggers several of the regulatory "extraordinary circumstances" exceptions to CEs, thus legally requiring the preparation of an EA or EIS.

3. Evaluating Offshore Fracking in a Programmatic EIS

If offshore well stimulation is proven safe, future offshore fracking should be evaluated through a Programmatic EIS (PEIS), similar to that currently being prepared by the California BLM. A PEIS is appropriate for assessing potential well stimulation in the Santa Barbara Channel, and would provide an opportunity for public participation and consultation with other state and federal agencies. Subsequent to a PEIS, DOI should still ensure that appropriate site-specific NEPA review is conducted for all exploration plans, APMs, and APDs.

4. Conduct Consistency Review for All Offshore Fracking Proposals

DOI should not wait for the California Coastal Commission staff to finish its own review of fracking to initiate overdue consistency processes under the CZMA. Instead, DOI should require operators to submit their fracking proposals (existing and proposed) to the Coastal Commission for consistency review.

5. Comply with the Endangered Species Act and Marine Mammal Protection Act

The Santa Barbara Channel contains extremely valuable habitat for numerous species listed as threatened or endangered, including blue, humpback, and fin whales, southern sea otters, and white and black abalone. Many of these imperiled animals receive overlapping but separate protections pursuant to the Marine Mammal Protection Act. DOI should ensure rigorous compliance with the Endangered Species Act, including section 7 consultation requirements, as well as requirements under the Marine Mammal Protection Act, prior to approval of any proposals involving fracking or other forms of well stimulation.

The Marine Mammal Protection Act-as well as the Endangered Species Act- require protection of great whales and other creatures that share our Channel. Currently federal oversight is failing that standard. © Erin Feinblatt

6. Review and Revise Clean Water Act Permit

Though recently revised, the current Clean Water Act permit regulating wastewater discharges from offshore California platforms does not specifically addresses frac waste streams such as flowback, and regulators were largely unaware that offshore fracking was even occurring during the revision process. If the general NPDES permit is found to be inadequate for addressing the unique impacts posed by fracking chemicals, the EPA should consider adopting individual permits for those platforms where fracking is being performed, in order to directly address chemicals that are outside the scope of what is authorized by the current permit, and either establish effluent limits for these chemicals or deny discharge altogether.

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8. Yergen, Stepping on the Gas, supra.
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28. Id.
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30. BSEE specifically requested that EDC narrow the scope of its FOIA request to exclude the practice of acid treatments due to its common usage. In order to receive documents regarding fracking as quickly as possible, EDC narrowed the scope of its request, and is now researching offshore acidizing in greater detail. See March 22, 2013 email from EDC to BSEE narrowing scope of FOIA request (on file with author).
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36. Channel Islands National Marine Sanctuary, Sanctuary Information, http://channelislands.noaa.gov/drop_down/mission.html.
37. Id.
38. Establishment of Marine Reserves and a Marine Conservation Area within the Channel Islands National Marine Sanctuary, 72 Fed. Reg. 29,208 (May 24, 2007).
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48. GRAHAM, supra at 20.
49. California v. Norton, 311 F.3d 1162, 1166 (9th Cir. 2002).
50. EASTON, at 203–04.
51. See EASTON, supra, at 8–9, 203–04.
52. Norton, 311 F.3d at 1166.
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123. U.S. HOUSE OF REPRESENTATIVES COMMITTEE ON ENERGY AND COMMERCE MINORITY STAFF, CHEMICALS USED IN HYDRAULIC FRACTURING 1 (2011).
124. Id. at Exhibit 2, at 19–20.
125. February 22-23, 2013 emails between BSEE and EPA staff (received through FOIA). When BSEE initially asked the EPA whether fracking fluids are covered under the general permit for well treatment fluids, the EPA claimed that fracking fluids may require additional provisions outside of those offered by the permit, which includes effluent limitations developed in 1993, long before the rise of shale fracking. Staff later reversed his opinion after consulting with EPA HQ.
126. David R. Baker, In-state Oil Fields Don't All Meet Standard, SAN FRANCISCO CHRONICLE (February 10, 2013); David Roberts, 10 Reasons Why Fracking for Dirty Oil in California is a Stupid Idea, GRIST.ORG (March 18, 2103).
127. Harold F. Upton. Coastal Zone Management: Background and Reauthorization Issues. CONGRESSIONAL RESEARCH SERVICE (Sept. 29, 2010).
128. Id.
129. Linda Krop, How States Can Affect Federal Deepwater Port LNG Licensing Decisions: A Case Study Involving the Deepwater Port Act and the Coastal Zone Management Act, 5 GOLDEN GATE U. ENVTL. L. J. 227, 236-237 (Fall 2011).
130. Id. at 237.
131. Steve Blank, In Defense of Unreasonableness—Saving the California Coast. HUFFINGTON POST (July 2, 2013).
132. See generally Sierra B. Weaver, Local Management of Natural Resources: Should Local Governments Be Able to Keep Oil Out?, 26 HARV. ENVTL. L. REV. 221 (2002).
133. 16 U.S.C. §1456(c)(3)(A).
134. Chang and Dearing, Oil Companies Frack in Coastal Waters off California, supra; Allison Sider. Scale of West Coast Fracking Probed, supra.
135. Statement of California Coastal Commission Deputy Director Allison Dettmer, August 15, 2013 CCC Meeting, Santa Cruz, California. Video archive available at: http://www.cal-span.org (beginning at approximately 40:30).
136. Id.
137. Dennis Hevesi. Peter Douglas, 69, Sentry of California's Coast, Dies. NEW YORK TIMES (April 9, 2012).
138. 16 U.S.C. § 1531 et seq.
139. 16 U.S.C. § 1536(a)(2).
140. 16 U.S.C. § 1361 et seq.
141. 16 U.S.C. § 1362(18)(A)(i), (ii).
142. 16 U.S.C. § 1371(a)(5).
143. NATIONAL COMMISSION ON THE BP DEEPWATER HORIZON OIL SPILL AND OFFSHORE DRILLING, supra, at 122.
144. CAL. PUB. RES. CODE § 3160(a).
145. Id. §§ 3160(a), 3160(a)(4).

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March 23, 2016

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Bureau of Safety and Environmental Enforcement
760 Paseo Camarillo
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RE: Programmatic EA of the Use of Well Stimulation Treatments

To Whom it May Concern,

Please accept the following comments on the Programmatic Environmental Assessment (EA) of the Use of Well Stimulation Treatments (WST) on the Southern California Outer Continental Shelf., which are hereby submitted by Santa Barbara Channelkeeper.

Santa Barbara Channelkeeper is a local non-profit environmental organization dedicated to protecting and restoring the Santa Barbara Channel and its watersheds through science-based advocacy, education, field work, and enforcement. While it is encouraging that well stimulation techniques for offshore oil development are being reviewed, this analysis falls short of providing the complete evaluation that is necessary to protect public health and natural resources as it incorrectly concludes that the existing National Pollution Discharge Elimination System (NPDES) permit provides sufficient coverage to address WST fluids.

The EA indicates that because the NPDES permit uses a multifaceted approach to monitor discharges to the marine environment, the discharge of WST fluids will have "no significant impact." However**, due to lack of monitoring requirements for specific WST constituents, the unknown toxicity of WST fluid constituents, and the lack of coordination between existing monitoring and WST activities, this approach fails to adequately monitor impacts from WST fluids**.

The current NPDES permit (General Permit No. CAG280000) does not provide adequate monitoring of WST fluids in the event that they are discharged as non-commingled waste. As outlined in Table 7 of the permit, such monitoring is only required once per treatment, and only includes documentation of volume of discharge, free oil (number of times sheen is observed), and concentrations of oil and grease. Monitoring for WST fluid constituents is not required.

While most WST fluids will be mixed (commingled) with produced water before being discharged, offshore platforms are only required to monitor discharged produced water for a limited set of constituents. Appendix B of the NPDES permit outlines the required constituents and measuring frequency for specific platforms. Appendix D includes an additional list of constituents for which certain platforms have conducted Reasonable Potential Determinations (RPD) and for which end-of-permit sampling must be conducted. However, none of the constituents listed in Table 4-12 of the EA, identified as the most common hydraulic fracturing components, are included in

the NPDES permit monitoring lists. Nor were any of the constituents listed in Table 4-12 analyzed during the RPD process. Therefore, the NPDES permit currently exhibits a critical monitoring gap with respect to WST fluids.

In addition to specific constituent monitoring, Whole Effluent Toxicity (WET) tests to evaluate chronic toxicity are also mandated under the NPDES permit. However, WET tests are only required at most quarterly, and likely only conducted once per year at this stage of the permit. As the EA acknowledges, WST fluids are likely to only be present in the discharged effluent for up to 10 days after use. Due to the infrequency of sampling it is unlikely that an effluent grab for a WET test would actually contain WST fluid. While WET tests theoretically are meant to capture constituents that are not specifically monitored under the NPDES permit, due to limited sampling frequency, the required WET testing is inadequate to verify that WST fluids are not contributing to chronic toxicity.

Moreover, as the EA acknowledges, many constituents of WST fluids lack toxicity data, and therefore potential effects on marine life within the mixing zone are not fully understood. We disagree that presumed dilution rates provide adequate assurance that toxicity will not occur. The NPDES permit included additional monitoring via the RPD analysis specifically to verify that produced water is unlikely to cause water quality impairment. Such analysis, using actual chemical data, is similarly needed before WST fluids can be determined to be not significantly impactful.

It is clear the current NPDES permit does not adequately monitor the impacts of WST fluids, and therefore the EA cannot conclude the NPDES permit is sufficient to justify a "no significant impact" determination. As such, Channelkeeper respectfully requests that a more thorough environmental review be completed and recommends that the moratorium on WST operations continues until an adequate review is finalized.

Thank you for the opportunity to comment on the Programmatic Environmental Assessment of the Use of Well Stimulation Treatments on the Southern California Outer Continental Shelf. We appreciate your attention to the issues and concerns we raise and trust you will more adequately analyze impacts for your final environmental review. Please feel free to contact me via email at ben@sbck.org or telephone at 805.563.3377 ext.3 should you have any questions.

Sincerely,

Ben Pitterle
Watershed and Marine Program Director