Alaska Department of Environmental Conservation

Assessment of Alaskan Marine Species for Toxicity Tests

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I. Executive summary, conclusions, and recommendations

The standard toxicity test protocols for marine organisms expose a warm-water test species to test chemicals. Most tests are done at room temperature (25^{o} C). For colder water, for example the typical Alaskan marine water temperature of 4^{o} C, there are no standard toxicity test protocols. Many of the standard test protocols can be emulated, by following all the standard procedures, and reducing the test water temperature to the desired colder temperature. The test temperatures relative to Alaskan waters, however, are likely to be fatal to most standard test species. This paper summarizes a literature search and interviews with Alaskan marine biological experts in an effort to identify Alaskan species that would be suitable for toxicity testing in cold water. The toxicity test protocols considered were primarily those of the Chemical Response to Oil Spills: Ecological Effects Research Forum (CROSERF). CROSERF is a research group composed of laboratories from government, academia and industry dedicated to improving laboratory research on the ecological effects of chemical agents used in oil spill response [8]. Those CROSERF protocols were designed to test the toxicity of oil and oil dispersants following a spill of oil in the marine environment. Also considered in this paper is the EPA's Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to West Coast Marine and Estuarine Organisms (EPA West Coast) [16], and some more general textbook toxicity tests [1, 2, 3]. While the CROSERF tests focus on the effects of chemical oil dispersants to pelagic organisms, the EPA West Coast tests consider nearshore and estuarine events.

Most modern marine toxicity experiments are done with animals in the immature or larval stages of development, typically the first two or three weeks after hatching. This is because the larval stages are sensitive to toxic chemicals, sometimes 10 or 100-fold more sensitive than the adults of the same species. Most Alaskan species only produce young once a year. This usually takes place during the spring bloom, the period of increasing sunlight in mid-spring. The exact timing and duration of the spring bloom varies from year to year. This means the larvae of most Alaskan species are available for only a few weeks. This leads to two broad choices for conducting toxicity tests on larval stages of

Alaska species: either most toxicity testing must be planned for sometime in a three month window in the spring, or an Alaskan species must be made available from a culture facility. Both choices have drawbacks, primarily drawbacks related to scheduling uncertainty in the first case and expense in the second case. For the first choice, the research team must be prepared well in advance of the spring bloom to begin the tests at a moments notice, while staying "on hold" waiting for egg production and hatching to begin. Once hatching begins, all the various research procedures must be completed in a few weeks. One critical mistake may delay the testing a full year. Working with small laboratory animals is filled with opportunities for such mistakes. For the second choice, culturing a new species requires research and development. Keeping marine species alive can be technically challenging, especially with larger species, but in general is quite feasible, given sufficient resources. The greater scientific problem is the induction of gamete (sperm and egg) production and spawning, such that larvae may be continuously produced or reliably produced on demand.

Included in the choices above is consideration of the location of the test facility. The University of Alaska Fairbanks (UAF) provides an economical location since analytical equipment and personnel are located in Fairbanks. Not all tests can be done at UAF, especially if the test procedure requires large amounts of seawater or quick access to the ocean.

Conclusions and Recommendations

The following are the conclusions and recommendations regarding a marine species for toxicity testing that may be applicable to Alaskan conditions. Regarding the order of listing, the least expensive and most relevant to the CROSERF dispersed oil procedures are listed first. All six species listed below, however, may be useful for certain types of toxicity testing and all should be considered for future studies.

1. Test the topsmelt (*Atherinops affinis*) in water as cold as practical. Topsmelt belong to the family Atherinidae, which is the most common family used in marine toxicity testing. No members of this family, however, are native to Alaskan waters. The topsmelt is cultured at colder temperatures, and larvae will likely survive down to

12 ^{o} C. Tests at lower temperatures have not been reported, although 8 ^{o} C is a likely lower limit of larval survival. Perform a series of temperature tolerance tests with the topsmelt. Begin by receiving larvae at 15 ^{o} C from a commercial culture facility, then decrease the water temperature 2 ^{o} C per day and observe the lowest temperate at which the larvae appear normal. Then run negative controls (without any test chemicals) and positive controls with the reference toxicant, copper chloride, according to standard EPA protocols. The rational for this recommendation is the commercial availability of topsmelt, its widespread use as a test species, and the current culture of this species at a temperature 10 ^{o} C lower than other commerically available species. This option will have moderate costs and conforms to the CROSERF protocols exactly, except for the reduced water temperature.

2. Culture a sea urchin species, most likely the purple urchin, *Strongylocentrotus purpuratus*, found in Alaska. Collect and culture the organisms initially at the UAF Seward Marine Center. (The green urchin, *S. droebachiensis*, may be easier to collect, and could also be used.) Experiment with methods of transporting urchins to UAF to determine the best transport methods. Perform negative and positive controls using both of the EPA's standard test methods, the embryo-larval development test and the fertilization test. The sea urchin test methods are well-known and widely accepted. They are excellent methods of testing for both successful fertilization and early development, and are considered sensitive tests of early life stages. The technology of making urchins spawn is available. The closely related species *S. franciscanus*, the red urchin, is harvested in southeast Alaska. Gonads of the red urchin are a delicacy in Japan. There is a green urchin fishery in Kodiak, also for the Japanese market. In the natural environment, urchins are eaten by the sea otter. Urchins are benthic or nearshore animals, but are relevant to dispersant studies because their gametes are dispersed into the water column and are known to disperse widely. This option is probably of moderate expense and conforms to the EPA West Coast toxicity protocols exactly, except for the water temperature, which will be colder in the Alaskan tests.

3. Coordinate with the Qutekcak Shellfish Hatchery (QSH) in Seward and obtain sperm and eggs from shellfish that QSH is spawning for commercial uses. The Pacific

oyster, *Crassostrea gigas*, (also known as the Japanese oyster) is a standard EPA West Coast test species, and this is the species most cultured by QSH. There is an excellent chance that sufficient gametes can be obtained as a by-product of QSH's normal operations. Run negative and positive controls, per EPA protocols. Timing of the shellfish protocols indicates that the laboratory work must be done in Seward. While gametes from shellfish may be available from time to time from QSH, the culturing of shellfish is a major technical undertaking. It is unlikely that it will be economical to culture shellfish for toxicity testing as a stand-alone operation. It may be possible to work with QSH to produce gametes on demand, but this would be an interruption of QSH's normal commercial activities and may be expensive. If the control experiments have good success, the economic aspects of this course of action may be perused further with QSH. Shellfish are benthic and most are nearshore species. This option may be of moderate expense and conforms to the EPA West Coast toxicity protocols exactly, except for the water temperature, which will be colder in the Alaskan tests. Expenses may depend on details of coordination with QSH.

4. Mysids and copepods are small crustaceans and species of both orders are used in standard toxicity tests. Both are found in the plankton and are relevant to dispersed oil studies. They are widespread and relevant to studies of effluent and other nearshore toxicity issues. They have a relatively short life, 90 to 120 days, and reproduce all year. They would have to be cultured, but it is likely that once cultured, they would produce larvae continuously. While mysids and copepods may be found all year, they are much more common during the spring bloom. At other times of year, a particular species may not be found. While the expense to culture either mysids or copepods would not be as great as the expense for the fish species discussed below, it would still be substantial. In addition, because copepods and mysids are so small and have such varied early life stages, skilled microscopists and taxonomists will be required to sort the animals prior to and during culturing. Readily available fresh seawater is desirable, but it is possible that, once a culture colony is established, culturing might be continued at UAF using artificial seawater. The selection of the particular species for culturing would require more study, with

the most likely prospect being a species with which other Alaska researchers have experience. This option is probably significantly more expensive than the first three listed. The mysid tests conform to the CROSERF protocols except for the water temperature, which will be colder in the Alaskan tests.

5. Pink salmon fry are available from hatcheries in the spring and could be tested. The only disadvantage, besides timing, is that the fry are no longer larvae and are expected to be relatively resistant to toxic chemicals. The fry would need large quantities of fresh seawater and therefore it is probably not practical to test salmon fry at UAF. This option will be of moderate expense, but conforms to neither the EPA West Coast nor the CROSERF protocols.

6. If the funds were available to establish a culture of an Alaska fish for use in toxicity testing, there are many possibilities. All would be new technology. The herring has the advantages of social and ecological relevance, but in general, a smaller species such as tidepool sculpin, sandlance, or members of the gunnel or prickleback families would be less expensive to maintain. Besides the initial problem of survival of the fish, technology would have to be developed to induce spawning on demand. Induction methods typically involve manipulations of photoperiod, water temperature, nutrition, and sometimes exogenous chemicals. Regardless of the species chosen, it may not be possible to induce spawning in northern species. Species from warmer climates sometimes spawn several times each year in nature, so these may be more amenable to induced spawning in the laboratory. Northern species generally have a very narrow window for spawning and may be less amenable to laboratory induced spawning. On this basis, the tidepool sculpin has a wide range and may be the best choice for a culturing experiment. This option is the most expensive of the six, but if successful will permit testing according to CROSERF, EPA West Coast, and other standard toxicity test protocols.

II. Introduction

II.A. Preliminary information

Contract INE 97.73, between the Institute of Northern Engineering (INE) of the University of Alaska Fairbanks (UAF) and Alaska Department of Environmental Conservation (ADEC) is titled: Evaluation of Toxicity of Dispersants and Dispersed Oil to Alaskan Marine Organisms. This report is submitted to fulfill Task 5 of that contract, Assessment of Other Alaskan Species for Toxicity Tests. This report takes advantage of INE's experience completing Task 3 of the contract, Toxicity Tests on Alaskan Tanner Crab Larvae. That toxicity testing was done according to testing methods of the Chemical Response to Oil Spills: Ecological Effects Research Forum (CROSERF). For the crab, this involved capturing gravid adult crabs in early January, maintaining them in holding tanks in UAF's Seward Marine Center (SMC) until March and April for egg development and larval hatching, and completing the toxicity testing in the narrow time window before hatching ceased, about 4 weeks. The many chemical samples required by the CROSERF procedures had to be shipped from SMC to UAF for analysis. The short time available for testing and the long lines of communication between UAF and SMC motivated the change in Task 5 from immediate testing of alternate species at the SMC to a literature search and investigation of alternate methods that would permit the animal care and rearing and the analytical work to be done at the same facility, preferably UAF. Also, the search was expanded to identify a cold-region species that might provide larvae or the other immature life stages, for use in toxicity testing methods other than the CROSERF methods.

II.B Why are we interested in dispersed oil?

In the event of a large oil spill, decision makers must determine if the spill should be treated by application of oil dispersants. Without dispersion, the oil, most of which remains on the surface, may contaminate marine epifauna, such as whales and birds. Wind and currents may result in large deposits of oil on surface features such as beaches, which may permit the further contamination of land fauna. Dispersants dissolve oil on the water's surface, then natural surface energy mixes the oil and dispersant into the water column. While this removes most of the oil from the water's surface, the resultant oil and dispersant is then available to contaminate the pelagic (sub-surface) environment. The decision to apply dispersants to an oil spill requires a knowledge of the toxicity of the undispersed oil to pelagic marine life occurring via natural dispersion, versus the toxicity of the oil-dispersant mixture. Understanding the toxicity of the dispersant alone is also useful to decision makers, because during an oil spill response, not all the dispersant will contact oil.

II.C Why are we interested in test species?

The Chemical Response to Oil Spills: Ecological Effects Research Forum (CROSERF) is a working group composed of individuals from Federal and state governments, academia, and industry dedicated to improving laboratory research on the ecological effects of chemical agents used in oil spill response[8]. Toxicity studies for several brands of dispersant on several types of crude oil have been performed. None of these studies were done on cold-water species and few were done with Alaskan North Slope crude oil (ANS crude), the most prevalent oil cargo in Alaskan waters. The composition of ANS crude oil can differ substantially from other crude oils and the physical behaviors of crude oil in cold water will differ from its behavior in warm water. The marine life will also vary as a function of temperature. Alaskan marine life will be acclimated to colder water temperatures, as low as 4^0 C, compared with most laboratory test species that are acclimated at 25^0 C. Ongoing research sponsored by the ADEC is studying the effects of Corexit 9500 dispersant and ANS crude on Tanner Crab larvae. Other ongoing ADEC sponsored research is focusing on the physical effects of ANS crude in cold water [9].

Most of the earlier CROSERF work was conducted using warm-water species. The testing temperatures were 25^o C. Typically the animals were cultured by the testing laboratory or were bought from commercial culturing facilities. This earlier CROSERF work has provided a body of knowledge about relatively few test species. The selection of a new test species will serve to enhance CROSERF work by extending the toxicity database to include new test species

This portion of the study focuses on the evaluation of other Alaskan marine species for toxicity testing. While the primary intent is to evaluate Alaska marine species for dispersant-oil toxicity testing, the species selected might also be pertinent to the testing of other xenobiotic chemicals in a cold-region marine environment.

III. Selection Criteria for Alaska Test Species

III. Introduction, ecological risk assessment

The possible adverse consequences of an action on the environment can be evaluated via a procedure known as an environmental risk assessment. Introduction of a stressor, such as a chemical oil dispersant, into the environment is an example of an action that can be evaluated by an ecological risk assessment. The two main data gathering phases of a risk assessment are the exposure assessment and the exposure-response evaluation. The exposure assessment examines the concentration to which the naturally occurring organisms in the environment will be exposed, that is, the environmental fate of the chemical in question. An exposure-response evaluation (or stressor-response profile, or dose-response curve) evaluates the likelihood of adverse ecological effects at various plausible concentrations of the stressor.

While the goal of the risk assessment is to evaluate the entire ecosystem, exposure-response testing is only practical with a few selected test species. The death or disability of selected species in laboratory tests is the assessment endpoint used in ecological risk assessment. The general methods and guidelines for selecting assessment endpoints in environmental risk assessment are available in standard texts [10] and EPA documents [7]. Generally the species used are socially recognized, ecologically relevant, susceptible to the chemical being tested, and manageable in the laboratory or observable in the field. The manageability in the laboratory, for the purposes of this project, must be compared to the standard CROSERF protocols.

III.A Socially recognized

Social recognition, in this context, refers to the public's acceptance of the risk assessment in general or the species selected as the endpoint. It is important that the testing can be used to support management decisions based on values and organisms that people care about [7].

The most readily accepted ecological endpoint would be an effect that was perceived to harm the public economically. The death of commercially or recreationally important species is a readily acceptable endpoint. This also requires consideration of habitat and lower trophic levels. In Alaska, both commercial and sport fishing are economically important.

Species that are protected by law, such as rare or endangered species, would be socially relevant, but these are usually not available for laboratory toxicity tests. The selection of species that are the prey of commercially or recreationally important species are also socially recognized, although their importance must be emphasized according to their role in the food chain. The selection of charismatic species, such as whales and eagles, is generally not logistically possible, but the relationship of the endpoint species to these charismatic species might bring public acceptance to the risk assessment.

III.B Ecological relevance

EPA states, "Changes in ecologically relevant endpoints can result in unpredictable and widespread effects [7]." The selected species should represent some ecological function that would be impaired by the chemical, i.e., are not rare or transient species. Small animals are food for larger animals. Young animals are both a food for larger animals and the means of propagation of their own species. In general, species with a long life are more sensitive to toxic chemicals than rapidly reproducing species.

III.C Susceptibility to known or potential stressors

The CROSERF procedures envision the application of dispersant to oil in an open marine environment, that is, not in nearshore wetlands, shallow water or small protected bays. Further, the dispersant or dispersant/oil mix will leave the surface and enter the water column, but this is typically in the upper few meters of the water column. Hence, the relevant endpoints must be pelagic species. It is important to realize that several important benthic (bottom dwelling) species have immature life stages that are planktonic life stages, and may be considered pelagic during that life stage.

The best input to the decision making process involves assessment of the most sensitive of the ecologically relevant endpoints that will be exposed to the stressor. That is, if the test or indicator species is ecologically relevant, it means that the depression of that species will certainly have an important effect on the ecosystem. Since typically there are many such ecologically relevant species, it is most economical to perform the endpoint on the most sensitive of those species.

Relative sensitivities of certain species to various classes of chemical toxicants are known. It is sometimes assumed that the sensitivity of a test species is representative of the particular class or phyla that the species represents. "A fish is a fish" is generally true for adults of many marine species. That is, for acute toxicity tests, the adults of many fish species are approximately equally sensitive [1]. While in general there is a high correlation between related species, some species may be much more sensitive to a particular class of compound, and there is no *a priori* means of detecting such sensitivities without substantial biochemical data.

For immature life stages, there is less information available. Typically the young are more sensitive than adults, although there may be other sensitive life stages, such as migration or shell molting. For many species that disperse sperm and eggs into the water, the gametes may be the most sensitive. Also, some species have a particularly sensitive season, when a food shortage or an abundance of predators make the species more sensitive.

Regarding application of dispersants to an oil spill, it will not be known what season the spill may take place. The worst case would be spill at the time the most sensitive life stages were present.

III.E Laboratory viability and practicality

CROSERF exposure regime and alternates.

The matters of laboratory viability and practicality must be evaluated relative to the exposure apparatus and exposure regime planned. For dispersed oil, the exposure apparatus and exposure regimes use to evaluate the stressor-response relationship are methods of the field of aquatic toxicology. The CROSERF procedures have been standardized for exposure to chemical dispersants alone, the water accommodated fraction (WAF, which includes both the oil dissolved in water and the oil otherwise present beneath the surface, i.e., micro-droplets), and the chemically enhanced water accommodated fraction (CE-WAF, which again includes micro-droplets, micelles of dispersant, oil, and a dispersant/oil combination). The Alaskan test species selected, however, might be used for toxicity testing other than for CROSERF protocols, that is, the stressor-response portions of the ecological risk of other chemicals might be more appropriately tested using procedures other than CROSERF. In the following paragraphs, the CROSERF procedures are described, then alternates to the CROSERF procedures that are plausible for uses of Alaskan species in aquatic toxicology are presented. The discussion of individual species selected for consideration will include what alterations to the standard CROSERF procedures would be required, if a candidate test species is otherwise desirable, based on social and ecological relevance.

Description of the CROSERF regime

The two main CROSERF protocols are called the "flow through" or "spiked" exposure and the "continuous" exposure. Both tests last 96 hours. The CROSERF flow through protocol is designed around special 250-ml, glass flow-through chambers [11]. These chambers were originally built by The University of California Santa Cruz (UCSC) and are loaned to various CROSERF participating laboratories. The CROSERF flow through procedure is used to simulate the brief high exposures of marine organisms to dispersant treated oil, following a spill in the marine environment. Wind and currents aid natural dispersion in the exponential decrease in concentration of CE-WAF to which the organisms are exposed. Note that in aquatic toxicology the words “flow-through” chamber usually has a different meaning. In the typical flow-thorough arrangement, the purpose of the flow-thorough system is to supply oxygen and remove wastes from the organism holding chambers. In that use, the test chemical is continuously added to the influent supply so that the organisms are exposed to a constant volume of contaminant. For that reason, the CROSERF flow-through exposure regime is called “spiked” exposure.

The other CROSERF procedure is “continuous exposure” whereby the test water is changed once every 24 hours, but each water change has the original concentration of test chemical. That is, the test animals are exposed to approximately a constant level of test chemical for 96 hours. The continuous procedure is very common in aquatic toxicology, so it is useful to correlated CROSERF findings with those of other, non-CROSERF researchers. The continuous exposure procedure does not mimic exposures from a chemically dispersed oil spill. Recently, the need for the continuous exposure procedure has been questioned and its use is no longer recommended for CROSERF dispersed oil studies [12].

Alternates: chamber size

Both CROSERF procedures are done with 250 ml containers, this is compatible with 5 to 10 individual test organisms per chamber. The small size of the chambers minimizes waste material and chemical use. This chamber size is about the minimum that can permit visible animals adequate swimming room, but limits the CROSERF procedures to small animals and would exclude species such a pink salmon fry and herring fry.

If it was not critical to following the CROSERF flow-through procedure with the UCSC glass exposure chambers, it would be possible to use larger animals. One general principal is that test animal should be as unstressed as is practical. Larger chambers are common in aquatic toxicology testing and would be required to test adult fish, since overcrowding causes stress. For continuous exposures, a chamber size of 0.5 to 0.8 gram of organism per liter of water is a general rule of thumb to avoid overcrowding for static/renewal [1]. Which would limit the size of animal in CROSERF chambers to about 0.1 to 0.2 g for an individual or 0.01 to 0.0.04 g for a test of 5 to 10 individuals. For flow through exposures, 0.5 to 1.0 gram of organism per liter per day is the minimum flow recommended.

Alternates: test regimes

General

All the CROSERF protocols, both spiked and continuous, are 96-hr acute tests. This conforms to the general exposure scenario of rapidly decreasing exposure concentration with time, the exposure reaching low concentrations in a few days. There are many ASTM and EPA toxicity tests for marine organisms that, other than the small CROSERF chamber size, are the same as the CROSERF continuous exposure regime. If these tests can be designed to expose the organism to declining concentrations, they might serve the ultimate purpose of dispersant testing with the spiked exposure as well.

Water

Most of the standard ASTM and EPA tests are done in 25^{o} C water, typically with a +/- 3^{o} C allowance. This is approximately room temperature, which is convenient for many purposes including simplicity of equipment. The Alaska species will be tested at a colder temperature, 4^{o} to 8^{o} C, and this will require the entire test be done in a cold room, or that portions of the test apparatus be chilled.

Life stage

Most of the acute 96-hr tests are done with the most sensitive life stage available, usually larvae or other immature stages of development. Life cycle tests are most appropriate for general toxicity but are usually considered chronic tests, rather than acute, and are more expensive. Testing of the toxicity of the test chemical to gametes, sperm and eggs, is very appropriate to spawning species. These tests take less time than the life cycle tests and are usually considered acute tests.

III.F <u>The criteria of comparability</u>

Several lists of commonly tested species for saltwater toxicity tests are found in Table 2 of Rand [3]. ASTM 729, *Standard Guide for Conducting Acute Toxicity Tests on Test Materials with Fishes, macroinvertebrates, and Amphibians* [2], Section 10.1, encourages the use of standard species: "If an objective of the test is to increase the comparability of results or increase information about a few commonly used species, or both, the test should be conducted with a species listed in Table 4. These species were selected on the basis of availability; commercial, recreational, and ecological importance; past successful use; and ease of handling in the laboratory. Their use is encouraged to increase the comparability of results and availability of much information about a few species rather than a little information about many species. If a desired species is unavailable, a species from a listed genus should be used." In the referenced ASTM table, the lowest test water temperature listed is 12 ^{o} C, (54 ^{o} F) with most tests at 17 ^{o} or 22 ^{o} C.

The approach used in this assessment is as follows: a list of likely Alaskan species to test was made based on personal communications from acknowledged Alaskan experts. A second list was formed by comparing the species and genera from the lists of standard (warm water) tests and review compendiums of Alaska species to see if species of the same genera are present in Alaskan water. The Alaskan species that are selected as being comparable will then be examined individually for social, ecological, and oil spill relevance, laboratory viability, and changes to the standard CROSERF protocols that might be necessary.

IV. Taxonomy of Plausible Test Species

The tables that follow integrate information from two main sources: first, lists of standard species that are commonly used in toxicity testing, most of these require warm water; and second, individual species that were recommended by various Alaskan researchers and ecologists, although not necessarily aquatic toxicologists. The tables indicate the taxonomy of each species, that is: phylum, class, order, family, genera, species, so that an approximate evaluation may be made regarding the how closely related various test species are. As noted earlier, if standard test species can not be used, species from the same genera are recommended.

Several large groups of species were not analyzed :

- Members of the plant kingdom, such as algae and macrophytes (seaweed). The testing of these is new technology and seldom used [3],
- Cnidarians: hydra, jellyfish, sea anemones and corals. Not usually considered socially or economically important, and
- Annelids, i.e., marine worms, not pelagic and not usually considered socially relevant.

Some species are not obviously relevant, but are included:

- Although the CROSERF procedures are written around a pelagic species, oysters and mussels are benthic or nearshore fauna, but their germ and larvae are pelagic, and
- Echinoderms, such as sea urchins and star fish are not usually considered socially or ecologically important, but there are many standard tests of life cycle toxicity that use these species and Alaskan urchins have been used commerically.

IV.B.1

Crustaceans (large: shrimp, crabs, lobsters)

SHRIMP

In the tables below, "AK" refers to a species recommended or used by Alaskan scientists. The other species are EPA or ASTM standard test species.

Phylum: Arthropoda (arthropods)					
Class: Crustaceans					
Order	**Family**	**Genus**	**Species (common name)**	**AK?**	**Ref.**
Decapodia (decapods)					
SHRIMP (Subclass malacostraca)					
suborder pleocyemata	Palaemonidae	Palaemon	adsperus		3
		Palaemon	macrodactylus		3
		Palaemonetes	pugio(grass shrimp)		2,3
		Palaemonetes	vulgaris(grass shrimp)		2,3
		Palaemonetes	intermedius(grass shrimp)		2
	Hippolytidae	Eualus	suckleyi (Kelp shrimp)	AK	
	Pandalidae	Pandalus	jordani		2
	Pandalidae	Pandalus	danae (Dock shrimp)	AK	2, 17
	Pandalidae	Pandalus	borealis (Pink shrimp)	AK	17, 18
	Pandalidae	Pandalus	hypsinotus (Coonstripe)	AK	17
suborder Dendrobranc-hiata	(Penaeid shrimp)	Penaeus	aztecus (brown shrimp)		2,3
	(Penaeid shrimp)	Penaeus	duorarum (pink shrimp)		2,3
	(Penaeid shrimp)	Penaeus	setiferus (white shrimp)		2,3
	(Penaeid shrimp)	Penaeus	stylirostris		3
	Caridea (Sand shrimp)	Crangon	septemspinosa (& other spec.)		2,3
	Caridea? (Bay shrimp)	Crangon	nigricuda		2

Crustaceans (large: shrimp, crabs, lobsters) continued

Crabs and lobsters					
CRABS class crustaceans					
Order	Family	Genus	Species	AK ?	Ref.
Decapodia sub(or infraorder Brachyura)	Canceridae	Cancer	irroratus (Rock crab)		3
	Canceridae	Cancer	magister (Dungeness)	AK	19
	Canceridae	Cancer	productus (Red)	AK	3
	Majidae	Chionoecetes	bairdi (Tanner)	AK	19
	Lithodidae	Paralithodes	camtschaticus (King)	AK	17
	Portunidae	Callinectes	sapidus (blue crab)		2,3
	Portunidae	Carcinus	maenas (Green crab)		2
	Gapsidae	Hemigrapsus	(several), “shore crab”		2
	Gapsidae	Pachygrapsus	(several), “shore crab”		2
	?	Uca	(several) (fiddler crab)		3
LOBSTER					
Decapodia	Nephropidae (lobster)	Homarus	americanus		2

IV.B.2

Crustaceans (small: mysids and copepods)

Phylum: Arthropoda (arthropods) Class Crustaceans					
Order	**Family**	**Genus**	**Species**	**AK?**	**Ref.**
Subclass Copepoda (copepods)					
Calanoida		Acartia	clausi		2
		Acartia	tonsa		
			(several)	AK	
Harpacticoida		Tigriopus	brevicornis		2
			(several)	AK	
MYSIDS Malacostraca subclass Peracarida					
Mysidacae Suborder Mysina	Mysidae	Mysidopsis	bigelowi		2
	Mysidae	Holmesimysis	costata		16
	Mysidae	Archaeomysis	grebnitzkii	AK	4,5
		Mysidopsis	almyra		2
		Mysidopsis	bahia		8
	?	Acanthomysis	pseudomacropsis	AK	25

IV.A.3

Other invertebrates

Phylum: Molusca Class: Pelecypoda (bivalves: clams, oysters, mussels)					
order	**family**	**Genus**	**Species**	**STD/AK**	**ref.**
Ostreoida (suborder. Ostreina)	ostreidae	Crassostrea (Atlantic oyster)	virginica		2
		Crassostrea (Japanese oyster)	gigas	AK	2
		Mercenaria (hard clam or Quahog)	mercenaria		2
Mytiloida	Mytilidae	Mytilus (blue mussel)	edulis (trossulus in AK and NW)	AK	2
Class: Gastropoda, Subclass Prosobranchia (snails and abalone)					
Archaeogastrop oda Suborder Pleurotomariina	Haliotidae	Haliotis	rufescens		16
Neogastropoda Suborder: Rachiglossa	Nucellidae	Nucella	lima	AK	
Phylum: Echinodermata (echinoderms: sea stars, urchins) Class: Echinoidea)					
Arbacioda	Arbaciidae	Arbacia	punctulata (Atlantic purple urchin)		1
Subclass Euechinoidea Order: Echinoida	Strongylocent rotidae	Strongylocentrotus	purpuratus (Pacific purple urchin)	AK	16
Subclass Euechinoidea Order: Echinoida	Strongylocent rotidae	Strongylocentrotus	droebachiensis (green urchin)	AK	4,5
		Strongylocentrotus	franciscanus (red urchin)	AK	
Clypeasteroida Suborder: Scutellina	Dendraster-idae	Dendraster	excentricus		16

IV.C **Phylum: Vertebrate**

Class: Pices

Subclass: Teleostei (bony fish)

Family	**Genus**	**Species**	**Common name**	**Notes**	**Ref.**
Order: Atheriniformes					
Atherinidae	Menidia	(several) (M. menidia used for dispersant res.	Silverside	family not AK	2, ,13, 14, 15
Atherinidae	Menidia	beryllina	Silverside (CROSERF)	family not AK	2, 13,14
Atherinidae	Atherinops	affinis	Topsmelt	family not AK (Aquatic Biosystems cultures cold)	Used in dispersant tests.
Order: Clupeiformes					
Clupeidae (Herrings)	Clupea	harengus (pacific herring C. pallasi)	Herring	found in AK	2 13, 14
Order: Cypriniformes					
Cyprinidae	Cyprinodon	variegatus	Sheepshead minnow	family not AK	2,13,14
Cyprinodontidae	Fundudlus	heteroclitus	Mummichog	family not AK	2, 13,14
Cyprinodontidae	Fundulus	similis	Longnose killifish	family not AK	2, 13,14
Order: Gadiformes					
Gadidae	Theragra	chalcogramma	Walleye Pollock	species found in AK	21, 25
Order: Gasterosteiformes					
Gasterosteidae	Gasterosteus	aculeatus	Threespine stickleback	species found in AK	2,13,14
?	Lagodon	rhomboides	Pinfish	genus not AK	2
?	Leiostomus	xanthurus	Spot	genus not AK	2
Aulorhynchidae	Aulorhynchus	flavidus	Tubesnout	found in AK	21, 25
Order: Perciformes					
Ammodytidae	Ammodytes	hexapterus	Pacific Sand Lance	found in AK	13, 14
Embiotocidae (surfperchs)	Cymatogaster	aggregata	Shiner perch	not found north of Wrangle	2, 13, 14
Stichaeidae	Spp.		(Pricklebacks)	24 species in AK	14

Class: Pices
Subclass: Teleostei (bony fish) (continued.)

Order: Perciformes (cont.)					
Stichaeidae	Anoplarchus	purpurescens	cockscomb prickleback	found in AK	21, 25
Pholidae (gunnels)	Spp			5 species in AK	14
Pholidae	Pholis	laeta	Crescent Gunnel	found in AK	21, 25
Order: Pleuronectiformes (flat fish)					
Bothidae	Citarichthys	stigmaeus	Sanddab	south to SE AK only	2, 13
Bothidae	Paralichthys	dentatus	Flounder	genus not AK	2, 13,14
Bothidae	Paralichthys	lethostigma	Flounder	genus not AK	2,13,14
Pleuronectidae (Halibut family)	Platichthys	stellatus	Starry flounder	found in AK	2, 13,14
Pleuronectidae (Halibut family)	Parophrys	vetulus (or vetula)	English sole	found in AK	2, 13,14
Order: Salmoniformes					
Salmonidae	Salvelinus	malma	Dolly Varden	found in AK	13, 25
Salmonidae	Oncorhychus	gorbuscha	Pink salmon (all AK salmon in this genus)	found in AK	13, 25
Order: Scorpaeniformes					
Cottidae	Oligocottus	maculosus	tidepool sculpin	species found in AK	2, 13, 14
Cottidae	Myoxo-cephalus	polyacantho-cephalus	Great Sculpin	species found in AK	21, 25

V.0

Discussion of species

V. General

Environmental toxicity testing is done with the most sensitive life stage available. For aquatic toxicology, this is typically the larval life stage, that is, shortly after hatching. For most Alaskan waters, all the life forms are synchronized to the “spring bloom.” The timing of the spring bloom varies with year and location, but is typically between March and May. This bloom is primarily dependent on the sunlight and the stability of the water column, not the temperature. At depth, waters are the coldest at this time. During the spring bloom there is a great increase in the amount of photosynthetic algae. This increase in numbers of producers at the lowest trophic level provides food for the first level of consumer species, either small animals or the larval stage of larger animals. These in turn provide food for the large species. In colder waters most species reproduce only once per year, and these species time the hatching of their eggs to coincide with this spring bloom. The implications of this for cold regions toxicity testing are that the larval stages will only be available during this spring bloom. For toxicity testing at other times of year, testing is limited to species that are not on this cycle, or species which are amenable to having this cycle perturbed in the laboratory.

The idea of freezing developed eggs, then thawing them when larvae were needed for toxicity testing is attractive because the cost of maintaining a frozen stock is much smaller than that of a live culture facility. The freezing of fish eggs for mariculture has been attempted and some success has been reported [24], but this new technology is not well developed. For Alaska species, it is clear that freezing eggs for storage would be a major research project in itself. Therefore, freezing of eggs will not be considered in the analysis that follows. If the state of the art improves, so that basic techniques are known, the freezing of eggs for future testing should be revisited.

Culturing small laboratory animals is common technology. For aquatic species, a source of fresh sea water is generally required. Artificial sea water is available, but for most culturing applications, large quantities of water are needed. For the culturing of an

Alaskan species to be useful for toxicity testing, the animals must not only be amenable to surviving in a culture facility, it is also necessary that they may be induced to spawn on certain schedules. For some animals, especially small fish, copepods, and mysids, this is not complex technology. In order to induce them to spawn on a particular schedule, however, would take a major project of one or perhaps several years.

V.A. Shrimp

1. Availability and General Information

There are five genera of shrimp listed as common for toxicity tests*: Palaemon, Palaemonetes, Penaeus, Crangon, and Pandalus.* Of these five genera, only *Pandalus* and *Crangon* have species commonly found in Alaskan Waters.

The genus *Pandalus* is well represented with 9 species of family Pandalidae mentioned by Kozloff [5]. Both *P. jordani* and *P. danae*, listed in ASTM procedures [2] are found in the northwest. Three additional *Pandalus* species, not in the standard lists: *P borealis*, *P. hypsinotus* and *P. goniurus*, have been recovered in Lower Cook Inlet [18]. There are many species of family Crangonidae, with 7 of Genus *Crangon* listed in Kozloff [5] but none of these are mentioned as standard test species. Crangon are found in the sand of shallow water environments [4]. Clearly members of the genera *Crangon* and *Pandalus* are found in Alaskan waters, and any of these would be similar to standard test species.

2. Exposure Methods

Tests of shrimp larvae consist of capturing ovigerous (egg bearing) females, clipping their chelipeds (appendage with claws) with fine scissors, and maintaining the shrimp in tanks. The fertilized eggs soon hatch releasing larvae, and the testing is done on larvae [3]. Once the larvae hatch, standard 96-hr exposure tests can be done (see below), so the CROSERF procedures could be used without modification.

3. Practicality

The Alaskan shrimp species: Coonstripe shrimp (*Pandalus hypsinotus*), dock shrimp (P. *danae*), and kelp shrimp (*Eualus suckleyi*) were used to test their sensitivity to drilling muds [17]. The method used was to collect gravid shrimp, isolate the various species into flow through tanks, and separate the larvae. Each test began with "stage I larvae, 0-3 days old." The tests may have lasted longer, but were "ended before first molt because of high natural mortality with first molt" [17] (the first molt takes place at an age of about 7 days). The water temperature was 5.6 ^{o} C; the larvae were not fed. Alaskan shrimp can be used for testing, when gravid shrimp are available.

4. Social relevance

The shrimp is well known to the public and likely to be perceived by the public as an important animal to investigate. Depending on the species chosen, the shrimp might also be economically relevant.

5. Ecologically relevant

All the shrimp species are important in the food chain. Some care is required in selecting the species so that a rare or transient species is not selected.

6. Relevant to dispersant investigations or other investigations.

The shrimp may be benthic (bottom dwelling) but the larvae are pelagic (free swimming) Some species are only found near shore or in limited locations. The species selected should be have a wide range.

7. Recommendations

With some further selection, there are several species of shrimp that are suited for toxicity testing of dispersants and other marine chemicals. Gravid Alaskan shrimp are not available commerically. They must be collected prior to each experiment, and held until they release larvae. Since the shrimp are only gravid during the "spring bloom," collection is highly seasonal. Shrimp could be cultured at the SMC, but it would take a major effort, perhaps one person half time while the shrimp are maintained. With this however, it is not certain that the shrimp can be induced to spawn at other than their natural times during spring bloom. In that case, there is no advantage to culturing them. There is no clear advantage to using shrimp over crab.

If testing of shrimp is required, several items could be researched further:

- Develop a schedule for each species of the usual hatching season at likely collection locations.
- Determine the species that are reliably collectable, based on historical data.

V.B. Crabs and Lobster

Crabs

1. Availability and General Information

Of the three species of the family Cancridae, genus *Cancer*, that have been used in standard toxicity tests, two species are common in Alaska, the Dungeness crab and the Red crab. *C. magister*, the Dungeness crab, is common and an important fishery. *C. productus*, the Red crab is also common, but not commercially fished because of its hard thick shell. The other families used in standard toxicity tests are not present in Alaska waters, except for several members of the genus *Hemigrapsus*, the shore crab, which are not relevant to dispersants and not likely to be economically or socially important and are not considered further. Family Nephroidae, lobsters, are not found in Alaskan waters.

In addition the Tanner Crab and King Crab have been used in Alaska for toxicity testing. The Tanner Crab, *Chionoecetes bairdi*, belongs to the family Majidae, and the King Crab, *Paralithodes camtschaticus*, belongs to the family Lithodidae.

2. Exposure Methods

The earlier work with Alaska crab species indicated the following procedure will work: Gravid females are collected in December or January and maintained in a mariculture facility with running seawater. The larvae are released from the female in the spring.

The larvae are separated from the mothers when they are 0 to 24 hours old and exposed in CROSERF or other chambers. The crab larvae are fed algae or diatoms and survive well.

3. Practicality

Toxicity tests with crab larvae are straightforward and have been done with Alaska species both by the U.S. Fish and Wildlife group at Auke Bay and the UAF group at the SMC. The crabs are seasonal, even though they are in a culture, the eggs reach maturity and hatch about the same time. This hatching time is not known in advance, however, and there is about a three-month window during which the experimenters must be prepared to start work and complete the work in 3 to 4 weeks.

4. Social relevance

All the Alaska species are harvested and perceived by the public as important. The Red crab slightly less so.

5. Ecologically relevant

All are ecologically relevant. The king is a deep water species.

6. Relevant to dispersant investigations or other investigations.

The larvae are pelagic and all the Alaska species can be used. The larvae (actually called zoae) fit 5 to 10 in a CROSERF chamber, but are still visible.

7. Recommendations

The techniques with crab larvae are well established. Any of the four species: Red, Dungeness, Tanner, and King could be used in CROSERF or other acute toxicity tests. The only limitation is the timing and the need to maintain the gravid females for several months. Hence the animal maintenance and chemical exposure work could not be done at UAF.

V.C. Mysids

1. Availability and General Information

All mysid species mentioned in the standard toxicity testing references [1,2,16] are warm water species. Mysids, sometimes called “opossum shrimp,” are common in plankton and other marine environments. A species common in British Columbia and the Northwest is *Archaeomysis grebnitzkii*. The mysid, *Acanthomysis pseudomacropsis* has been used for toxicity testing in Alaska [25]. The warm water mysid species are easily cultured and many tests can be done with them through their 28-day life and reproduction cycles [2]. Selecting and rearing a cold water species might be a difficult task. The standard EPA West Coast tests uses the mysid *Holmesimysis costata*, which is “common intertidal among eelgrass and algae on sandy or rocky beaches; found near river mouths and in bays and lagoons, but generally restricted to higher salinities [4,16]."

2. Exposure Methods

The warm water mysids are common in aquatic toxicology and are used in many different types of tests, both acute and reproductive. The EPA West Coast test method has a procedure for testing of mysids that uses water as cold as 13^{o} C.

3. Practicality

Developing a cold water species will take considerable effort. The warm water procedures assume that gravid females can be collected as needed. The short life of the mysid requires that breeding continue all year. This is true in colder waters as well, but there are fewer individuals in the colder seasons. Finding sufficient adults for testing is not a sure thing. Identification of species is also difficult and requires a skilled taxonomist. It is likely that once a species is collected, it will be necessary to culture it through one or more generations to assure sufficient animals are available for testing.

These problems are similar to that of copepods, as discussed below. Use of the standard EPA West Coast test method mysid, *H. costata*, may be possible at colder temperatures. This mysid is available from commercial laboratories.

4. Social relevance

Mysids are not known to the general public. The importance of mysids in food chain of larger species would require some explanation.

5. Ecologically relevant

Mysids are an important part of the food chain. Some knowledge of the species selected would be required in order to assure it is not a transient or rare species.

6. Relevant to dispersant investigations or other investigations.

Mysids are part of the plankton and nearshore waters. They are used in dispersant investigations, although the species used to date have been estuarine species.

7. Recommendations

Since mysids are so common in aquatic toxicity testing, a cold water mysid would be credible to other scientists in aquatic toxicology and be a species for which many different testing regimes have been used. If funding were available, a stock of mysids could be cultured at SMC or other marine center with running sea water available. The following recommendations parallel those for the copepod below:

- Run a plankton trawl in the colder months and carefully review the species of mysid obtained. Separate into species and count the number of gravid females. Take the several species so obtained and maintain them separately in tanks in SMC. Feed the most appropriate food that is readily available. Sample periodically and assess the number of gravid females and assure the integrity of the species, and
- Run a ranging test, using the EPA West Coast protocol, in so far as practical. This test requires juveniles. These larvae could be obtained from the freshly collected females if sufficient quantities are obtained, or from the cultured group.

V.D. Copepods

1. Availability and General Information

Copepods are small crustaceans. The two genera common in toxicity testing, *Acartia* and *Tigriopus*, are not found in the Northwest or Alaska. There are several species of orders *Calanoida* and *Harpacticoida* found in Alaska. Because they are noted as being better laboratory species, only the order Harpacticoida will be considered.

2. Exposure Methods

There are many tests that can be done with harpacticoid copepods, including the standard 96-hour tests. Since egg sacks are produced every three days, multigenerational studies are practical. Both 7 and 12 day exposures have been used.

3. Practicality

Use of copepods would require first collecting, then culturing. Generally, adults must be collected because the immature life stages vary so much from the adult, separating is not practical until adulthood. In choosing a species, it is important to choose a species that is easy to distinguish. Further, copepods can be herbivorous, carnivorous or omnivorous. Food will be easier to find for the herbivorous species. Some species are common in most years, but not every year.

Rand has references to culturing copepods in laboratory but he notes many attempts fail, although harpacticoids are easier to rear than Acartia [3]. Using a 7-day test and counting the offspring will assure several stages of larvae have been exposed. A skilled microscopist is needed to count the copepods.

4. Social relevance

Herbivorous copepods are the first level consumers in the food chain. They are frequently found in the stomach of salmon smolt and are assumed important in the diet of young salmon.

5. Ecologically relevant

Care is required when selecting species that a rare species is not chosen. The common species are important in the food chain.

6. Relevant to dispersant investigations or other investigations.

Copepods are an important part of the plankton, and are a pelagic species and relevant to dispersants.

7. Recommendations

If funding were available, copepods could be cultured in the laboratory.

- Parallel to the recommendations for mysids above, a cold weather plankton tow should be done and species separated, counted etc.
- There is a good possibility copepods can be reared successfully in the lab, but more information is needed on the basic biology and identification of the species, nutrition, etc.

V.E.I Mollusks

0. General

The discussion of mollusks is divided into the major classes of mollusks, bivalves: clams, oysters, etc., and gastropods: snails-like animals.

I. Bivalves

1. Availability and General Information

Clams, oysters and mussels have long been used in aquatic toxicity studies. The two oyster and one clam species mentioned common in toxicity testing [2] are not native to Alaska nor the Northwest Pacific, but they have been introduced in some Northwest localities [4]. Japanese oyster (sometimes called the Pacific oyster) which is common in toxicity testing [2,16] has been introduced into Alaskan waters. It will not reproduce naturally in cold water, but commercial growers can import young oysters (spat) and settle them on empty shells (clutch) [4].

The mussel *Mytilus edulis* is used in toxicity testing [2,3] and is common in Alaska. There is some confusion regarding the name of the mussel species, *Mytilus edulis*. True *M. edulis* is probably only found on the Atlantic coast. The species reported as *M. edulis* in Alaska and the Northwest is properly *M. trossulus* [5]. *M. edulis* and *M. trossulus* are very closely related, certainly the same genus. The following assumes that all references in Kozloff [4,5] to *M. edulis* are *M. trossulus*, and are transcribed as *trossulus*.
M. trossulus, also known as the blue mussel or bay mussel, prefers quiet waters and estuaries where the salinity is relatively low. It attaches to rocks or wood piling.

The Qutekcak Shellfish Hatchery (QSH), co-located with the Seward Marine Facility, cultures shellfish spat (early life stages) for sales to Alaska Native mariculture operations. The species of shellfish QSH cultures or has been culturing include:
Littleneck Clam *(Protothaca staminea*)
Pacific Oyster (*Crassostrea gigas*)
Rock Scallop (*Crassadoma gigantea*)
Cockle (*Clinocardium nuttallii*)
Geoduck (*Panope abrupta*)
QSH could culture other shellfish, including mussels, but this currently lacks an economic basis. The Rock scallop and Geoduck are not noted as Alaskan species in Kozloff [5].

2. Exposure Methods
The shellfish are cultured (kept alive in a laboratory). Light, feeding, and water temperature cycles are adjusted to bring the shellfish to fertility. Eggs and sperm are collected separately, then mixed. The newly fertilized eggs, embryos, are placed in the test solution. After 48 hours the number of properly developed larvae is counted. This is typically a static, non-renewal test.

3. Practicality

There are standard ASTM and EPA test methods for both the Pacific oyster and the Blue mussel and the methods are similar. Culturing the shellfish probably requires a sophisticated facility with running sea water. It may be possible to culture shellfish for a short time in a UAF lab. Besides seawater, the shellfish required phytoplankton for food, and these must also be cultured. Blanchard and Feder [20] notes blue mussels developed gametes throughout the winter in Prince William Sound, including periods of freezing air and water. The main factors controlling reproduction and nutritional storage cycles are thought to be temperature and food availability. However, these cycles are apparently flexible, as mussels are able to adapt to individual environment quickly.

The QSH is set up and produces the embryos needed for these tests. The goal of QSH is not toxicity testing, so some modification to the QSH procedures are required in order to conform to the ASTM or EPA procedures. By scheduling the toxicity testing to conform to the QSH schedule, the test organism would be available, perhaps at no expense.

4. Social relevance

All the shellfish cultured at QSH are socially relevant, that is, they are being cultured commerically. Most are recognized by the public. The Blue Mussel is eaten by sea otters and other socially relevant species.

5. Ecologically relevant

The Pacific Oyster is not native to Alaska, and will not reproduce in cold water. It will live and grow in cold water. The mussel is native to Alaska and will reproduce in cold water, but is not cultured in QSH because there is no commercial demand for it. Blue mussels are in the food chain of many species

6. Relevant to dispersant investigations or other investigations.

Mussels and oysters are nearshore species. Their fertilization and embryonic development could take place pelagicly but most takes place in the nearshore. These shellfish tests would be more relevant to nearshore effluent issues, than oil dispersants.

Because the shellfish tests are so well established and because of the possibility of applications of dispersant closer to the shore, the shellfish tests are not irrelevant to dispersant studies.

7. Recommendations

Coordination with QSH is important, but if good coordination, both administrative and technical, is achieved, it may be possible to perform the shellfish tests using small amounts of sperm and eggs produced a byproduct of QSH's normal operations. In addition, there is some lab space in the QSH building. The standard ASTM and EPA tests are not simple and require personnel skilled in both shellfish culture and aquatic toxicity testing. In addition, it is unlikely that the tests could be done satisfactorily the first time. For relevant reproduction testing, the personnel would need experience with the test. Because it is not the most relevant tests to dispersants, the work involved is probably not justified for dispersant studies. On the other hand, for near shore pollutants, it may be one of the most relevant tests.

II. Gastropods.

1.Availability and General Information

The red abalone is a standard test species [16] but it is a warm water species, as are the other members of family Haliotidae. There is a "northern abalone," *Haliotis kamtschatkana*, which may be found in colder waters, but it was not recommenced by Alaskan researcher and may not be common [4].

Several Alaskan researchers have mentioned the use of the snail species *Nucella lima* which deposits a pouch of eggs in the intertidal zone. The pouches are available in the winter, in some locations. Inside the pouches are fertilized eggs, which develop into small snails. The pouch is sturdy and can be maintained in oxygenated seawater without food.

2. Exposure Methods

There are no established exposure methods for the snail

3. Practicality

This is a species that is available in the winter, at least in some locations in Alaska and the pouches could be transported back to UAF for testing. Although the pouches are available in the winter, in nature they hatch in the spring. It may be possible to accelerate this hatching, but this would be new technology. In addition, the pouches of several species appear similar and species identification may not be possible .

4. Social relevance

Probably not socially relevant.

5. Ecologically relevant

Snails are predators of clams and barnacles. Snails are eaten by birds. Snails are not known as a vital part of the food chain.

6. Relevant to dispersant investigations or other investigations.

Unknown

7. Recommendations

Do not investigate further.

V.F. Echinoderms

1. Availability and General Information

Sea urchins are commonly used in experimental biology and keeping a sea urchin colony alive in the laboratory is relative easy [17]. They are sensitive to pollutants [16,17]. The most commonly tested sea urchin,[2] the purple urchin, *Arbacia punctulata*, is a warm water species. Neither its genus nor family is found in the Northwest [4,5]. There is an urchin species, *Strongylocentrotus purpuratus*, also called the purple urchin, that is common to Alaskan waters and is a standard EPA West Coast test species [16]. A member of that genus, *Strongylocentrotus droebachiensis*, the Green Sea Urchin, has a wide distribution in northern waters. The advantage with *S. purpuratus* is that there is much comparative literature regarding toxicity.

The green sea urchin is abundant in rocky intertidal areas. *S. purpuratus*, the purple urchin, also has a wide range. The red urchin, *S. franciscanus*, is found in warmer Alaskan waters, typically south of Sitka. In nature, the eggs and sperm are discharged into the sea. If the eggs are fertilized, they develop into a distinctive planktonic stage, which later settle out.

The sand dollar, *Dendraster excentricus*, is also an EPA test species and is found in the Northwest in sandy areas, but perhaps not as far north as south-central Alaska. Other sand dollar species are found in Alaska. The focus here will be on the urchin, because more is known about the Alaskan species and the test methods are better known.

2. Exposure Methods

The EPA test is essentially a test of the toxicity of the test chemical to the sperm, eggs and the fertilization process. After a brief, 20-minute, exposure of the sperm, eggs are added and 20 minutes later the process is stopped and the material fixed and the number of fertilized and properly developed embryo counted. Spawning is induced with chemical injection.

3. Practicality

The urchins are generally collected, which may require scuba diving for collection of purple and red urchins, or green urchins may be collected at low tide. Fresh seawater is required but this may be supplied by artificial seawater. Following collection, the urchin may be transported to UAF. The urchins eat seaweed pieces and other detritus. Urchins have developed gonads throughout the year, so urchins may be a good candidate for induced spawning [26].

4. Social relevance

The sea urchin tests directly measure toxicity to the fertilization process and there are well established procedures for those tests. Also, the Alaskan species, *S. franciscanus,* the red urchin, is harvested. Gonads of the red urchin are a delicacy in Japan. There is an active green sea urchin fishery on Kodiak Island. The red urchin is larger than the green and its selection as a food item is likely related to its size rather than other characteristics.

5. Ecologically relevant

Urchins are a food for sea otters.

6. Relevant to dispersant investigations or other investigations.

Although adults of most species have maximum depths of 10 to 15 meters, the sperm and eggs are pelagic. And as noted above, sea urchin tests are excellent tests of toxicity to the fertilization process. The sea urchin fertilization test is quite different than the standard CROSERF tests.

7. Recommendations

The urchin test is not a CROSERF test, but it is a standard EPA test and is the best test of fertilization and may be quite feasible in UAF. We recommend getting a supply of urchins at a convenient time, arrange with SMC to care and feed them, then with a subset of these, examine transporting them to UAF and try culturing them and inducing spawning, etc. If so, perform ranging tests with reference toxicant. Temperature limitation of spawning is a separate experiment.

V.G FISH

V.G Introduction

Many species of fish have been used in aquatic toxicity testing and many others have been recommended by Alaskan researchers. Toxicity testing has been done on nine fish species which included an estimation of the median lethal dose of crude oil [25]. In interest of brevity, the discussion of most species is limited to the major technical disadvantages that would make it difficult for that species use in aquatic toxicity testing. In most cases, the disadvantage is that the adult fish must be cultured in order to obtain the larval or immature life stages needed for testing. Many of these fish could be cultured in a marine laboratory such as the SMC, but this would have a large time and money cost, and in general we deemed those species impractical. Some species are mentioned as possibilities for laboratory culture, because they have other advantages.

In general, all the fish are tested in the first week after hatching and would conform to the CROSERF protocols and similar ASTM and EPA test methods, so exposure scenarios for individual species are usually not discussed.

V.G 1: Standard test species:

Of the listed marine species [2] only the Threespine stickleback, tidepool sculpin, Starry flounder, English sole, and Herring are found in colder Alaskan waters. In addition, the Shiner perch and Sanddab are found in Southeast Alaska.

Threespine stickleback

1. Availability and General Information

The Threespine stickleback (*Gasterosteus aculeatus*) has two varieties listed by Wilimovsky [14], *G. aculeatus aculeatus*, and *G. aculeatus microcephalus*. Both species are found in Alaskan waters. Eschmeyer [13] notes they are anadromous, spawning mostly in fresh water. They are properly described as euryhaline rather than marine. They eat small pelagic organisms, mostly crustaceans, and small fish. The stickleback is eaten by fish, seals, and sea birds. Record size is 10 cm, but most are much smaller.

2. Exposure Methods

The stickleback is no longer used as an EPA test species, although it is still used by the Corps of Engineers for dredging permits. The older standard tests of the stickleback used adult fish, hence the CROSERF chambers would not work and larger tanks would be required. If larval fish were used, the CROSERF procedure would work.

3. Practicality

Sticklebacks could be collected in Alaska. Adult sticklebacks are available from a supplier in California.

4. Social relevance

Not well known.

5. Ecologically relevant

A part of the food chain for many species.

6. Relevant to dispersant investigations or other investigations

Yes, if cultured.

7. Recommendations

Adult sticklebacks have passed out of use for most marine aquatic toxicology testing because it was found that the adults were not a sensitive species, that is, they could survive higher concentrations of most toxicants than similar species. For that reason, the large investment required to culture sticklebacks in Alaska is not recommended.

Tidepool Sculpin

1. Availability and General Information

The tidepool sculpin is a nearshore species that commonly inhabit tidepools and the calmer intertidal areas [13, 21]. They are common and easy to collect. Maximum lengths up to 9 cm have been reported. Another sculpin, the great sculpin, has been used in oil toxicity testing [25].

2. Exposure Methods

Standard

3. Practicality

Easy to collect adults. Would require culturing to obtain larvae. In nature, their reproduction is keyed to the spring bloom.

4. Social relevance

Not a well known species, the ease of observation of the adults might give them some recognition.

5. Ecologically relevant

Their prevalence indicates they are probably in the food chain of many species, but this is not noted in literature.

6. Relevant to dispersant investigations or other investigations

The adults are common near shore and intertidal. They are known to prefer their home tidepools, hence they might not be considered pelagic.

7. Recommendations

Because this is a common test species and easily obtained, it would be a good candidate, if a culture facility were attempted. It is kept in aquaria [21]. Prior to selection, more literature searching should be done to determine if successful culturing has been done, albeit with warmer waters. The key to culturing successfully would be to determine if they could be made to spawn on demand, hence produce larvae as needed for toxicity testing. This would require development of new technology.

Flat fish

1. Availability and General Information

Two flat fish species are both common in marine research, the Starry flounder (*Platichthys stellatus*) and the English sole (*Parophrys vetulus*) and found in Alaska waters. They are different genera, but must be closely related species because a cross between the two species, the Hybrid sole, is noted. The English sole, also known as the Lemon sole, is important commerically [13, 21]. The Starry flounder is noted as a game fish [13]. The Sanddab is in the same family as two species that are found in colder waters, so it will not be discussed further.

2. Exposure Methods

Although listed as a common test species by ASTM, recent references to their use in toxicity testing were not found.

3. Practicality

Uncertain. Culturing would be required. Conversations with culturing facilities indicated that most flat fish, especially the Sanddab, were collected, not cultured, which indicates testing by others has not been done on larvae.

4. Social relevance

Flatfish have a high public recognition.

5. Ecologically relevant

Adult flatfish are bottom dwellers or benthic species. Many species spawn in shallow water and migrate to deeper water later.

6. Relevant to dispersant investigations or other investigations

Their habits make them not highly relevant to dispersants, which are assumed to be used in deeper water, since the eggs and larvae are deposited in shallow water. They would be relevant in general for effluent toxicity.

7. Recommendations

Because of their strong social recognition and the economic importance of closely related species such as halibut, either of these flatfish species should be a candidate, if culturing is a possibility. The adults are large, and this might make them less practical for culturing. A literature search and interviews may provide insight into details of their use.

Shiner perch

The Shiner perch family is not important in Alaska, so its warmer water habitat and lack of important do not invite further discussion.

Herring

1. Availability and General Information

Herring are seasonally common and have been used in toxicity testing in Alaska. Herring are usually found inshore in harbors and large estuaries during spawning. Spawning takes place in winter and/or spring (later in the north). Each female lays up to 125,000 eggs which are sticky and cling in masses to eelgrass, kelp, and fixed objects. The Alaska species is considered by some researches to be a Pacific population of the Atlantic herring, *Clupea harengus* [13].

Rice, et al., [22] used adult herring and eggs in several experiments. In one experiment the researchers started with adults in 1000L fiberglass tanks collected with purse seine. They also tested collected eggs from seaweed to which they are attached. They noted that "Feeding larvae...are killed by shorter exposures and lower concentration than the eggs or adults."

2. Exposure Methods

Many methods have been used, included larvae, which would fit the CROSERF procedures.

3. Practicality

Eggs are readily available in the spring. Eggs could be rapidly shipped to UAF or other locations for the experiments.

4. Social relevance

Important commerically and easily recognized by the public.

5. Ecologically relevant

Important species.

6. Relevant to dispersant investigations or other investigations

Spawning takes place between high tide line and depths of 36 feet [21]. The larvae remain in shallow water the first six months of life, then migrate to deeper waters. The adults are certainly pelagic and directly relevant to CROSERF. The larvae are more relevant to effluent pollution issues.

7. Recommendations

Herring are an excellent test species, the only drawback in the narrow time window when their eggs are available.

Sand Lance

1. Availability and General Information

Sand Lance is found is several habitats: offshore, in schools in channels , and buried in the sand nearshore. It is common in Alaska. Adults can grow to 8-10 inches. The sand lance is a long narrow fish. Sand lance (Pacific sand lance, *Ammodytes hexapterus*) is an important food for predatory fishes, sea birds and marine mammals. Habitat includes burying into the sand and swimming in schools [13, 21].

2. Exposure Methods

There are no reports of this species being used in toxicity testing.

3. Practicality

Adults are very common at some times and would be readily available by seining. At other times their availability may be more difficult. Details of its life cycle and spawning habits are not known.

4. Social relevance

Not a well known species, but their place in the food chain as salmon prey would help public accept them.

5. Ecologically relevant

The sand lance is frequently taken as food by Chinook and Coho salmon, lingcod, halibut, fur seals, and many other marine vertebrates, including birds [21].

6. Relevant to dispersant investigations or other investigations

The varied habitats of the sand lance would make it relevant to most types of aquatic toxicity testing.

7. Recommendations

Culturing the sand lance would be required in order to test larvae. The varied habitats of the sand lance might make it amenable to culturing, but this is unknown. It would be new technology, and this is not recommended unless more is know about the life cycle.

Gunnels

1. Availability and General Information

Three species of family Pholidae: *Apodichthys flavidus*, *Pholis laeta*, *P. ornata*, are noted in Alaska [21]. The most common, *P. laeta*, the Crescent Gunnel, occurs in the intertidal zone and tidepools, but also down to 55-73 meters. It has been used in Alaska toxicity testing [25].

2. Exposure Methods

Adults were used in testing of crude oil toxicity, reported in 1979 [25].

3. Practicality

Details of its life cycle and spawning habits are not known for all three species. The adults are noted to be easy to find intertidally by seining.

4. Social relevance

Not a well known species.

5. Ecologically relevant

Not known.

6. Relevant to dispersant investigations or other investigations

The varied habitats of the gunnels would make them relevant to most types aquatic toxicity.

7. Recommendations

Culturing the gunnel would be required in order to test larvae. It would be new technology, and this is not recommended unless more is known about the life cycle. SMC noted it would be a 1 to 2 year project to establish the reproductive biology and perhaps establish a culture. It may be that reproduction is light sensitive.

Pricklebacks

1. Availability and General Information

Many species of family Stichaeidae are found in Alaska waters: *Anoplarchus insignis, A. purpurescens, Chirolophis decoratus, C. nugator, Lumpenus maculatus, L. sagitta, Phytichthys chirus, Poroclinus rothrocki, Xiphister atropurpureus, X. mucosus,* are noted [21]. *A. purpurescens* has been used in Alaska toxicity testing [25].

2. Exposure Methods

A. purpurescens has been used in testing toxicity of crude oil [25].

3. Practicality

Details of its life cycle and spawning habits are not known for any of the species. They are noted as being found in trawl nets and some are available by this method.

4. Social relevance

Not a well known species.

5. Ecologically relevant

Not known.

6. Relevant to dispersant investigations or other investigations

The many species of Pricklebacks makes it likely that some of them would be relevant to aquatic toxicity testing.

7. Recommendations

Culturing the Pricklebacks would be required in order to test larvae. It would be new technology, and this is not recommended unless more is known about their life cycle.

Pink salmon fry

1. Availability and General Information

Pink salmon fry are available, at a certain time of year, from hatcheries.

2. Exposure Methods

Fry are to too large to fit in the CROSERF chambers, but have been tested in tanks. The fry are not a larval form, they almost one year old, and are expected to be relatively resistant to toxics. Because of their larger size, continuous flow-though type testing would be required. They have been used for toxicity testing in Alaska. Carls, et al., [23] used pink fry from hatchery, and implied they were put directly into seawater. Fry were maintained in 800L tanks then put in 65 L for testing.

3. Practicality

The fry are readily available. Directly from the hatchery they are still acclimated to fresh water and would have to acclimated to salt water. For the pink salmon, this is a rapid process, i.e., several days. Some hatcheries use a “net pen” towards the end of the hatchery process. The pens are placed in salt water. Fry from these pens would be acclimated to salt water and might be hardier. The fry would require large quantities of salt water and the testing would not be practical at UAF.

4. Social relevance

Highly recognized.

5. Ecologically relevant

Yes.

6. Relevant to dispersant investigations or other investigations

In general, salmon fry after they enter marine waters are a pelagic species and would be relevant for CROSERF testing. Their presence in nearshore waters is brief and hence they would not be relevant for chronic effluent toxicity.

7. Recommendations

The pink salmon fry make an excellent test species except for their seasonality and the fact that as a more mature life stage, they may be resistant. Dolly Varden have also been used for toxicity testing in Alaska, but provide no advantages over the pink salmon [25].

Topsmelt

1. Availability and General Information

Atherinops affinis is common in California but is found only north to southern British Columbia. They are included here for two reasons, first, they are a standard EPA West Coast test species [16] and second, commercial culturing facilities have some experience culturing this fish at low temperatures.

2. Exposure Methods

Nine to 15-day old larvae are tested in static renewal tests for the EPA methods, but the topsmelt have been used in a great variety of aquatic toxicity tests

3. Practicality

Larvae are readily available by fast air shipment from culture facilities in the lower-48.

4. Social relevance

Not well known outside scientific circles. In California, they are a very common species and are often among the most abundant species in California estuaries [16].

5. Ecologically relevant

Not relevant in Alaska.

6. Relevant to dispersant investigations or other investigations

In the warmer waters where it is found, its habits are similar to the herring, and it is likewise relevant to both dispersant and effluent testing.

7. Recommendations

Aquatic Biosystems (AB) raises topsmelt and drops their water temperature to 12.8^{o} C AB noted that adults do fine at 8-12^{o} C. They spawn at 16-22^{o} C. AB does not see a problem with shipping larvae at 15^{o} C nor with survival down to 12^{o} or 10^{o} C. Below 10^{o} C the larvae may become lethargic. A reasonable experiment would be to obtain larvae that have been shipped at 15^{o} C and drop the temperature 2^{o} degrees per day and observe fitness and mortality down to 8^{o} C or perhaps lower. (The test temperature for a cold water should be 4^{o} C, this may not be possible with the topsmelt.) An allied problem is the feeding of the larvae. The standard food, brine shrimp nauplii, dies at cold temperatures. The topsmelt larvae may or may not eat the dead nauplii, if not, alternate foods are required to feed the larvae for the 4 or 7 day tests. If the topsmelt larvae survive and are testable at a low temperature, positive controls, that is, exposure to a reference toxicant should be done.

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