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Applying Life Stage Sensitivity Data in Chemical Control Strategies for Invasive Animal Species

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PURPOSE: This technical note discusses how quantifying the relative sensitivity of different life stages of aquatic nuisance species (ANS) to chemical exposures may optimize control strategies. It is generally accepted that the early life stage of an organism is more sensitive than adults to chemicals. Therefore, it is intuitive that chemical control strategies designed to target sensitive life stages may provide more effective control and would require lower treatment doses, incurring lower product cost and risk for non-target species. Generation of chemical life stage toxicity data for different ANS is critical for the development of such an approach, providing supporting guidance for dosages used in field applications. Ultimately, the probability of survival of different life stages of ANS in the presence of a chemical control along with their ecology and demographic information could be utilized in a population model to provide projections of control efficacy. For this effort, life stage sensitivity data were generated using a model ANS to provide relevant information to develop such a control strategy; the invasive marine bivalve *Mytilus galloprovincialis* was exposed to the chemical stressors chlorine, copper, and a molluscicide, Bayluscide®.

BACKGROUND: As modern transportation has expanded, biogeographical boundaries have diminished, creating greater potential for the introduction of exotic species. New aquatic species can be introduced into U.S. waterways inadvertently (e.g., ballast water release, escape from aquaculture facilities) or intentionally (e.g., aquarium trade, biological control, introduction of species for aquaculture). A few species have potential to disrupt local ecosystems, fisheries, and infrastructure. Such invasions may directly impact U.S. Army Corps of Engineers (USACE) activities and responsibilities for construction and maintenance of harbors, ports and waterways, erosion control, management and stewardship of water resources, and wetland and coastal habitat restoration (Ray 2005).

Any species transported outside the boundaries of its native range has potential to become a nuisance species. Once unrestricted by evolved natural controls (e.g., predation, disease, parasites, competition, etc.) in their native range (Torchin et al. 2003, Wolfe 2002), such species may reach abundances impinging upon the above resources, particularly if demographic characteristics (e.g., reproductive fecundity, dispersion rates, growth rates, etc.) allow exponential growth and a population explosion (e.g., zebra mussels, *Dreissena polymorpha*). Successful invaders often tend to be abundant over a large range in their native region, have broad feeding and habitat preferences, wide physiological tolerances, short generation times, high fecundity, and high genetic variability (Erlich 1989, Williams and Meffe 1999). Although these characteristics are relatively easy to identify, it is difficult to predict which species pose the greatest threat. This is because most species are not ubiquitous in their native range and opportunities for introduction and subsequent likelihood of survival, exponential population growth, and damage of natural resources and infrastructure are difficult to assess or predict (Ray 2005).

Control measures for invasive bivalves fall into two broad categories: non-chemical and chemical (Claudi and Mackie 1994). Non-chemical methods include trapping, physical removal antifouling coatings, thermal and electric shock. Chemicals are an important component of integrated zebra mussel control programs, and can provide versatile, easy to implement, and cost-effective methods to deal with established infestations, and reduce new infestations (Sprecher and Getsinger 2000). While chemical treatment can be designed to protect whole systems, a major drawback is the requirement for safe discharge in compliance with environmental regulations. Management plans for invasive species are limited by local environmental, regulatory and financial constraints, and as such may focus on a small suite of sub-optimal control measures, including sporadic capture/trapping, attempts at exclusion or chemical treatment.

In the current study, the authors propose a control strategy that targets only the most sensitive life stages of an invasive species, which therefore requires significantly lower chemical exposures. While all species develop from immature to mature forms, some species (e.g., bivalve molluscs) pass through several developmental life stages (e.g., egg, larvae, adult) that may have vastly different chemical sensitivities. Larval zebra mussels have been reported to be more susceptible to chemicals than adults (Sprung 1993, Stoeckel and Garton 1993, Mackie and Kilgour 1994, Kennedy et al. 2006), although this may not hold true for all chemicals (Fisher et al. 1994). Consequently, various researchers have suggested that larvae are the best target for chemical control (Waller et al. 1993, Fisher et al. 1994, Kennedy et al. 2006), particularly since adult zebra mussels are less sensitive to many chemical exposures than early life stages, and can avoid exposure to aqueous application of some chemicals by terminating water intake via shell valve closure (Mersch et al. 1996, Kennedy et al. 2006) for periods up to 2 weeks (Sprecher and Getsinger 2000).

However, by definition, a chemical-based management plan targeting only the most sensitive life stage would provide ineffective control of more resistant life stages. Such management plans are unlikely to achieve short-term eradication of an ANS population, but instead impact recruitment. Thus, success criteria must be chosen carefully. One strategy is to depart from traditional short-term population eradication goals and describe efficacy in terms of (1) suppression of exponential growth (and associated damage) by controlling recruitment, and (2) eventual eradication by significant and sustained control of a chemically sensitive life stage. A common invasion dynamic is an initial high density, followed by a crash to low density (Schlesch 1930, Sebestyén 1938). The key rationale is that, while achieving complete eradication of an ANS population is rare, controlling recruitment by targeting the most sensitive life stages may diminish harmful population explosions that occur shortly after introduction. This approach is shown in Figure 1, where repeated treatment over a period of three years targets the recruitment of offspring, resulting in a significant decline in population rate increase. Effective control would be accomplished when the population rate increase is negative and adult organisms begin to reach their life expectancy. Such an approach requires joint application of ecotoxicology and population demography, coupling life-stage sensitivity data with a population model to allow predictions of invasive species control.

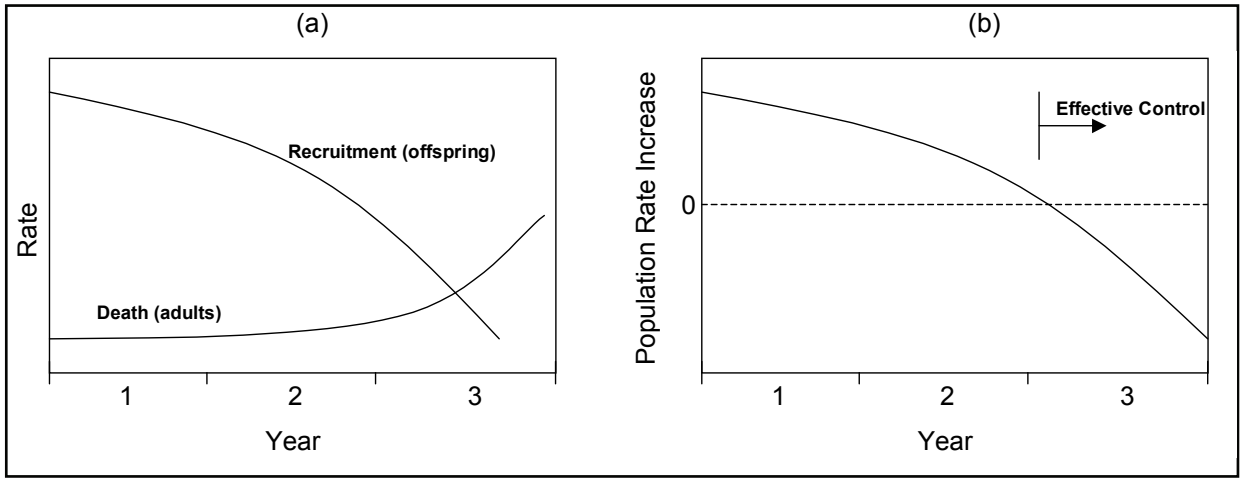

Figure 1. Hypothetical population response to a control where a more sensitive early life stage is targeted and adults naturally die off within 3 years (panel a), which ultimately leads to a decline in population growth (panel b).

Since it is difficult to assess success criteria such as larval recruitment for lower doses of chemical control applications, integration of matrix population models would be of great use to managers. Population models, specifically matrix models, have been commonly applied in the field of ecology for more than 25 years to describe population dynamics (Caswell 1989). Specifically, these models are applied for population projection when survival rates and birth rates are assumed to depend on age or developmental stage, which would be of particular use for mussel life stages where great differences in chemical tolerance are suspected. Such models have been used to project population trends of threatened and endangered species, applying different management parameters to determine if the measure resulted in an increase in population growth. Conversely, for ANS, the approach would be to model the population to extinction by manipulating the control parameters. Using matrix models, the population at any time-step is represented as a vector of age-specific or stage-specific population sizes. Only stage-specific models are considered, since the model species metamorphose into distinct stages, which may more accurately describe relative sensitivity. Given the limited budget and arsenal of control measures available for invasive species, managers would be well served by invasive species population models that predict a likely outcome of a control measure, or combination of measures, on the invading population. A matrix population model enables scenario testing capabilities and is described in more detail with a toxicological context in Bridges and Carroll (2000). Models could be used to test predictions of control efficacy under a number of realistic scenarios, including the proportion of each life-stage that must be eradicated to achieve eventual population extinction, and the effects of an influx of non-exposed animals into a target population on the long-term viability of that population. It should also be noted that other non-chemical control strategies (e.g., harvesting of adults by trapping) could be employed in scenario tests of the long-term population viability. For example, trapping of the Chinese mitten crab, *Eriocheir sinesis*, during pre-spawning migration (Chinese Mitten Crab Working Group (CMCWG) 2003) would select only adult crabs of a size range dictated by the trap dimensions, while the current study demonstrated that mussel larvae are much more sensitive to chemical

control than small adults, which are in turn more sensitive than large adults. Exploration of population models for this use is targeted for later years of this project.

To generate toxicity information to populate such models, the Mediterranean blue mussel, *Mytilus galloprovincialis*, native to the Mediterranean Sea, was selected as a model ANS. This mussel was introduced to both the West Coast of the United States and Hawaiian waters for aquaculture (Eldridge and Evenhuis 2002). It is also exotic to South Africa, Japan, Hong Kong, Korea, Australia, Mexico, and Canada (Branch and Steffani 2004) and was nominated as one of the "top 100 world's worst invaders" (Ray 2005). On the U.S. Pacific coast, its range extends from Penn Cove, Washington to San Diego, California, potentially resulting in interference with restoration of rocky intertidal habitats by crowding out native species (Ray 2005). This species was selected because of its current status as an invasive species of concern, our past experience in inducing bivalve gamete release *in vitro* (Kennedy et al. 2006), thus facilitating toxicity testing, and the existence of a standard testing method for a congeneric species, *Mytilus edulis* (American Society for Testing and Materials (ASTM) 2004).

The objectives of the first year of this project were to 1) quantify the toxicity of three chemicals to the early life stages of *M. galloprovincialis*, 2) quantify the toxicity these chemicals to three size classes of adult *M. galloprovincialis*, and 3) compare sensitivity data for larval and adult stages.

MATERIALS AND METHODS:

Test organisms. Adult *Mytilus galloprovincialis* were obtained from Carlsbad Aquafarms (Carlsbad, CA) or Penn Cove Shellfish Inc. (Penn Cove, WA). Two suppliers were used because (1) spawning success is variable between sources, (2) supplier water temperatures exceeded 20°C during experimentation (June to August), at which suitable gamete maturity may occur in only 0 to 15 percent of mussels (Hrs-Brenko 1971) and (3) to gauge potential inter-population sensitivity differences. Mussels were shipped overnight in coolers containing cold packs and were spawned within 2 hr of delivery, since mussels were observed to release gametes soon after they were placed into aquaria (this study[1]).

Spawning induction. Spawning was induced by thermal stimulation, with mussels taken from shipment containers and placed directly into individual 300-mL tall-form beakers containing 30 ‰ artificial seawater (ASW; Instant Ocean Sea Salt®, Aquarium Systems, Inc., Mentor, Ohio, U.S.A.) at 27 to 30 °C (ASTM 2004). Mussels were observed to spawn between 15 min to 3 hr after induction, at which time they were transferred to 16 °C sand-filtered natural seawater (San Diego Bay, Scripps Institute). Sperm and egg viability were assessed under a microscope prior to the use, and were pooled prior to fertilization when multiple organisms spawned.

Exposure chemicals. The toxicity experiments used concentration ranges of copper (Cu), chlorine (Cl_2), the proprietary molluscicide Bayluscide®, and a control (Table 1). Copper was selected because it is known to be toxic to molluscs and can be applied in open systems in the

[1] Personal Communication. 2005. G. Rosen, research scientist, Space and Naval Warfare Systems Center, San Diego, CA.

form of Cutrine-Ultra® to control algal blooms (Kennedy et al. 2006). Chlorine was selected because it has an extensive track record for controlling molluscs in closed industrial systems (Claudi and Mackie 1994). Bayluscide® was selected due to a previous study discussing its use in open waterways to control the sea lamprey, its high specificity for inducing toxicity in molluscs and rapid degradation in natural water and sediments (Dawson 2003). Chemical concentrations in control solutions were less than method detection limits.

Copper (Cu) exposures used reagent grade cupric (II) sulfate ($CuSO_4$, CAS 7758-98-7, 98.2 percent purity) made in deionized water to achieve solubility. Aqueous solutions were acid digested using EPA method 3010A (U.S. Environmental Protection Agency (USEPA) 1996), and analyzed using inductively coupled plasma – mass spectrometry (USEPA 1996). Chlorine (Cl_2) exposures used reagent grade sodium hypochlorite solution (NaOCl, CAS# 7681-52-9, 13 percent free chlorine) made in artificial seawater (ASW; Instant Ocean Sea Salt®, Aquarium Systems, Inc., Mentor, Ohio, U.S.A.). Actual Cl_2 exposures were measured in the highest four exposures at the start and termination of the 24-hr exposures. A 64- to 81-percent decrease in Cl_2 was observed over the exposure duration, due to volatilization of Cl_2 from the aqueous phase. Concentrations of Cl_2 were measured using an Orion 97-70 residual chlorine probe (Thermo Election Corporation, Beverly, MA, U.S.A.). Bayluscide® wettable powder (aka, Bayer 73, niclosamide; EPA Reg. No. 6704-87) exposures used 72.9-percent active 5-Chloro-N-2-chloro-4nitrophenyl)-2hydroxybenzamide compound with 2-aminoethanol (1:1) (Bayer Cropscience, Monheim, Germany) made in ASW. Concentrations were measured at the start and termination of the 24-hr exposures using a high performance liquid chromatography (HPLC) technique based on Dawson (1982).

Table 1. Nominal exposure concentrations (µg/L) used in early life stage exposures. Measured concentrations were used in determining toxicity reference values for copper and chlorine, while nominal values were used for Bayluscide®.

Copper	0.5	2.0	7.8	31.3	125	500		
Chlorine	0.2	0.6	2.4	10	39	156	625	2500
Bayluscide®	0.1	0.2	1.0	3.9	16	63	250	1000

Toxicity tests methods. Toxicity tests with the above chemicals were conducted in the laboratory using different life stages of *M. galloprovicialis*. Figure 2 is a schematic of these life stages.

The early life stage toxicity test method was based on previous *D. polymorpha* research (Fisher et al. 1994, Kennedy et al. 2006). Toxicity of the chemicals to *M. galloprovincialis* larvae was assessed using 24-hr lethality tests. The relative sensitivity of different life stages (pre-fertilization, post-fertilization, 24-, 48-, and 144-hr old larvae) was quantified by initiating chemical exposure at different times relative to fertilization (Figure 3). In pre-fertilization tests, unfertilized eggs were exposed to chemical treatments 15 min prior to sperm addition (i.e., fertilization occurred in the presence of chemical). In the post-fertilization tests, eggs and sperm were combined and 30 min were allowed for fertilization to occur before chemical dosages. In addition, some post-fertilization tests were continued to 168 hr to assess delayed effect. Tests used Costar Inc. (#3516) six-well plastic tissue culture plates (Corning Inc., Corning, NY), with

each well containing a total of 10 mL volume, and six replicate wells per treatment. Tests were conducted at 16 ± 1°C, an appropriate temperature for *Mytilus* species (ASTM 2004). Wells were loaded with an equal volume of an egg suspension, based upon an initial density determination (n = 3; CV < 10 percent). Controls (ASW) were conducted in parallel with mussels for each stage. All intact larvae were considered alive at test termination, since they were capable of motility after close observation. Inter-treatment survival was assessed relative to the average initial number of eggs per well.

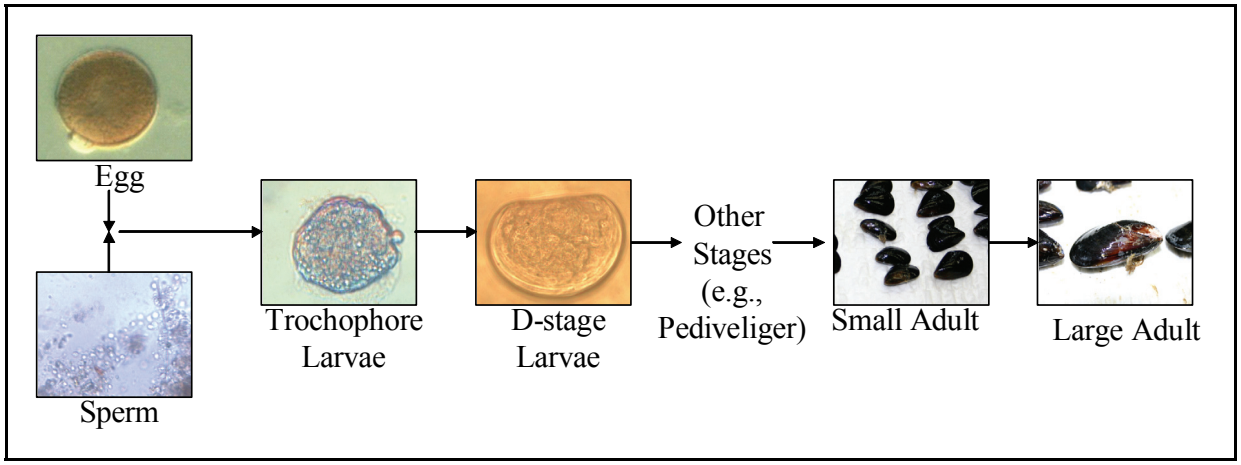

Figure 2. Chronology of the various life stages of *Mytilus galloprovincialis* tested. Eggs / sperm (< 1 hr old); Trochophore larvae (24 – 48 hr old); D-stage larvae (48 – 72 hr old, 144 - 168 hr old); small adult (1.5 – 2.5 cm); medium adult (2.5 – 5.0); large adult (5.0 – 7.0 cm).

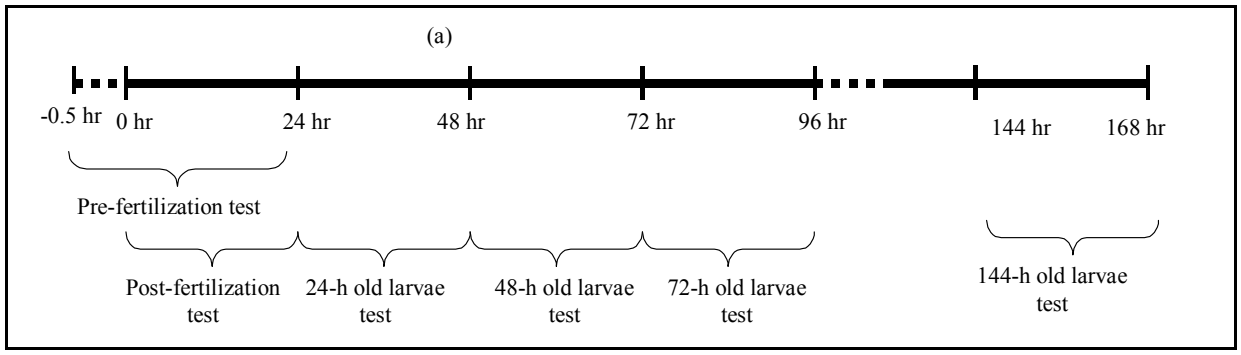

Figure 3. Staggered application of the 24-hr toxicity exposures of mussel larvae. The zero hour represents fertilization. Timeline is not drawn to scale.

Adult toxicity tests were conducted using 96-hr static renewal exposures (Figure 4). Organisms were shipped overnight from Penn Cove Shellfish Inc. (Penn Cove, WA) as described above and transferred to 20-L aquaria containing 30 ‰ ASW at 16 ± 1° C. Water was exchanged daily since animals spawned during the first 96 hr of holding. Mussels were grouped into three size classes; small (1.5 – 2.5 cm), medium (2.5 – 5.0 cm), and large (5.0 – 7.0 cm); size classes were exposed to five treatment concentrations of each chemical for sensitivity comparisons. Each treatment included three 1-L beaker replicates, containing five mussels each. Controls (ASW) were conducted in parallel with mussels for each size class. The solutions were aerated, and the

water was completely renewed each day. Survival and presence of filtering activity were assessed daily. Deceased mussels, identified if gaping and failing to respond to gentle prodding, were promptly removed. At test termination, mussels were removed from water and survival was assessed more thoroughly by teasing valves apart.

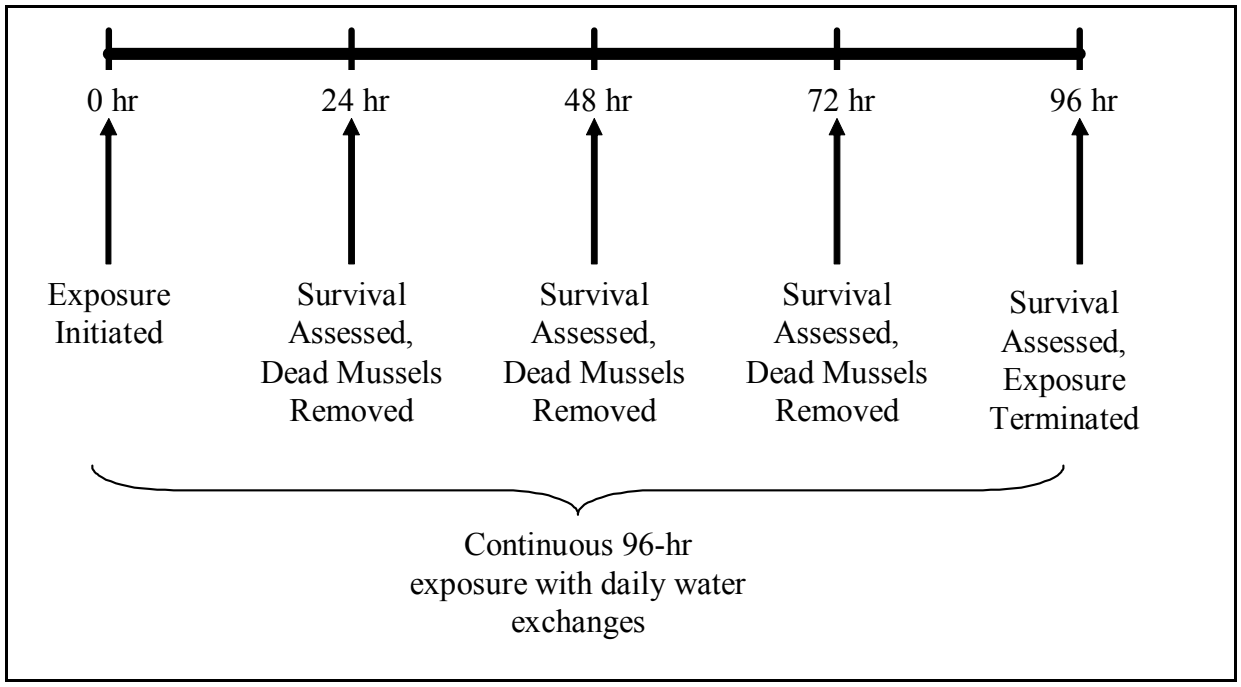

Figure 4. Schematic summarizing the 96-hr adult exposure. Timeline is not drawn to scale.

Data Analysis. Survival data were analyzed using maximum-likelihood probit analysis to estimate the 95-percent confidence limits for lethal concentration inducing mortality in 50 percent (LC_{50}) and 99 percent (LC_{99}) of exposed individuals for both adult and early life stages. Where the data failed to conform to maximum-likelihood probit analysis, Spearman – Karber probit methods were performed. ToxCalc© software was used for all analyses (Tidepool Scientific, McKinleyville, CA), with data arcsin square-root transformed and Abbott's correction used to account for control mortality. Point estimates were considered significantly different when 95-percent confidence limits did not overlap or, in cases where the program did not allow estimation of 95-percent confidence limits for a data point, when that data point did not fall within an estimated 95-percent confidence range. All toxicity data analyzed were based upon measured concentrations, with the exception of Bayluscide®, for which nominal values were used.

RESULTS AND DISCUSSION: The median and 99-percent lethality reference values for early life stages of *M. galloprovincialis* to Cu, Cl_2, and Bayluscide® are summarized in Table 2. Adult mussels were observed to actively avoid Cu and Cl_2 over 96-hr exposure by closing their valves. Such avoidance was also observed in the congeneric *Mytilus edulis* (Howell et al. 1984), the Asiatic clam (Tran et al. 2004), and zebra mussels (Kennedy et al. 2006) when exposed to Cu, demonstrating the broad ecological advantage of this behavioral response in bivalve mollusks. It may be concluded that these species have chemoreceptors that trigger a response to

Cu (e.g., Kraak 1992). Masilamoni et al. (2002) documented avoidance behavior by the green mussel, *Perna viridis*, when exposed to chlorine. However, avoidance was not as apparent in adult mussels exposed to Bayluscide®, which therefore is likely more effective than Cu and Cl_2 if targeting adults.

Table 2. *Mytilus galloprovincialis* toxicity reference values determined for early life stages exposed to copper (Cu), chlorine (Cl2), and Bayluscide®. Exposure period was 24 hr. Ninety-five percent confidence intervals, where available, are provided in parentheses. All concentrations are expressed in µg/L.

Chemical	Life stage	Population	Exposure (h)	LC_{50}	LC_{99}
Cu	Pre-fertilization	P	24	25.0 (0.2 - 67.5)	433.1 (171.2 - 29760.0)
	Post-fertilization	P	24	8.0 (0.4 - 23.5)	172.8 (88.7 - 391.9)
	24 hr	P	24	40.7 (37.0 - 45.0)	98.7 (82.9 - 126.0)
	48 hr	P	24	47.3 (41.3 - 53.3)	108.0 (93.9 - 128.4)
	144 hr	P	24	53.7 (42.6 - 62.9)	137.7 (122.2 - 160.3)
	Post-fertilization	C	24	34.2 (16.6 - 52.1)	160.5 (87.6 - 1767.2)
	Post to 168 hr	C	168	12.6 (12.2 - 12.8)*	NC
	24 hr	C	24	28.8 (12.7 - 40.8)	102.1 (59.3 - 5440.0)
Cl_2	Pre-fertilization	P	24	36.1 (24.6 - 44.6)	NC
	Post-fertilization	P	24	475.0 (NL)	3380.8 (NL)
	24 hr	P	24	500.2 (152.9 - 1039.1)	2454.3 (1164.5 - 14331.8)
	48 hr	P	24	680.4 (602.8 - 743.7)	NC
	144 hr	P	24	469.2 (390.2 - 615.0)	NC
	Post-fertilization	C	24	716.9 (682.8 - 752.8)*	NC
	Post to 168 hr	C	168	275.5 (244.4 - 310.7)*	NC
Bayluscide®	Pre-fertilization	P	24	12.1 (11.4 – 12.82)*	NC
	Post-fertilization	P	24	16.8 (15.8 – 17.9)*	NC
	24 hr	P	24	12.4 (2.2 - 24.5)	201.9 (82.8 - 4135.4)
	48 hr	P	24	125.0 (no limits)	NC
	144 hr	P	24	125.0 (no limits)	NC
	Post-fertilization	C	24	31.9 (31.7 - 32.1)*	NC
	Post to 168 hr	C	168	24.7 (24.5 - 27.0)*	NC
	24 hr	C	24	31.8 (31.6 - 32.0)*	NC

P = Penn Cove population; C = Carlsbad Aquafarm population; NC = Curve-fitting did not permit estimation of LC values;
* Calculated by Spearman-Karber method.

The Cu LC_{50} values for gametes prior to fertilization, and for 24-hr, 48-hr, and 144-hr old larvae were not significantly different (Table 2), ranging from 25.0 to 53.7 µg Cu/L. However, Penn Cove larvae exposed to Cu directly after fertilization demonstrated a significantly higher sensitivity, with an LC_{50} of 8.0 (0.4 – 23.5) µg Cu/L. The significance of this result is unclear and a similar trend was not observed for Carlsbad larvae. The LC_{50} value for larvae exposed at post-fertilization for 7 days (168 hr) was significantly lower than other stages, except for the 24-hr post-fertilization larvae of Penn Cove mussels. The LC_{99} values were similar for all life stages, ranging from 98.7 to 433.1 µg Cu/L. For 96-hr adult toxicity tests, LC_{50} (3,150 µg Cu/L)

and LC_{99} (157,400 µg Cu/L) values could only be generated for the smallest size class (1.5 - 2.5 cm), which indicated more than 60 and 360 times greater tolerance than larvae, respectively. This is especially significant, given that adults were exposed 72 hr longer than larvae. Due to lack of mortality, the 96-hr LC_{50} value for the larger adult size classes was assumed greater than 4,484 µg Cu/L.

All toxicity reference values for Cl_2 represent a pulse exposure, provided this chemical begins to volatilize from solution within a few hours. Research by Matisoff et al. (1990) suggests that larvae mortality occurs well within a few hours of exposure to Cl_2. The LC_{50} values for Cl_2 were similar for all larval stages after fertilization, and ranged from 469.2 to 680.4 µg Cl_2/L. However, Cl_2 exposures applied prior to fertilization resulted in a significantly higher sensitivity compared to all other early life stages (LC_{50} = 36.1 µg Cl_2/L). The significant difference between the pre- and post-fertilization exposures suggests that Cl_2 has a higher impact upon the gametes, fertilization, or cleavage than on the larval stages after the fertilization period. The LC_{50} value for larvae exposed for 7 days at post-fertilization was significantly lower than the 24-hr post-fertilization result, indicating a delayed effect, provided the majority of Cl_2 volatilized from solution after 24-48 hr. Lethality in 99 percent of larvae exceeded 2,000 µg Cl_2/L. No toxicity reference values could be generated for adult mussels during four pulse exposures of 7,500 Cl_2/L over 96-hr. However, mortality was below 50 percent for five different marine mussel species exposed to Cl_2 for 96 hr when the exposure to approximately 8,000 µg Cl_2/L was kept at constant concentration (Rajagopal et al. 2003). Thus, it can be suggested larvae are at least 10 to 200 times more sensitive than adults to the lethal effects of Cl_2, depending on which stages are compared.

A considerable difference in sensitivity to Bayluscide® between each life stage and between populations was evident when comparing LC_{50} values (Table 2). The Penn Cove mussels appeared more sensitive than Carlsbad mussels based upon post-fertilization and 24-hr-old exposures. In addition, D-stage larvae (> 48-hr old) featuring shell formation (LC_{50} = 125 µg/L), were significantly more tolerant than pre-fertilization, post-fertilization, and 24-hr-old larvae (12.1 – 31.9 µg/L), which lacked shells. Slightly greater sensitivity was observed with post-fertilization exposure following 7 days compared to the 24-hr post-fertilization result, as indicated by the lower LC_{50} value (Table 2). It was assumed that Bayluscide® degrades over this duration[1], although this was not quantified. Adult 96-hr LC_{50} values for the small, medium, and large size classes were 226 (186 - 379), 424 (300 - 599), and 500 (no limits) µg/L, indicating that 7 to 40 times more Bayluscide® is required to promote lethal effects in adults relative to larvae. An LC_{99} of 1,602 (950 - 7,501) µg/L could be generated only for the smallest adult size class.

CONCLUSION: This technical note contrasts the sensitivity of numerous *M. galloprovincialis* life stages to Cu, Cl_2, and Bayluscide® to generate data that could populate an ANS population model. The data presented imply that the early life stages of the model species were much more sensitive than adult stages. Ultimately, control practices targeting the most sensitive life stages could reduce the required treatment dosage and consequently cost and increase the margin of safety by applying a species-specific dosage of a particular chemical or technique, or combination thereof, based upon data generated in the laboratory. Such a strategy may aid in the

[1] Personal Communication. 2004. Betty Mitchell, B. L. Mitchell Inc., Leland, MS.

maintenance of water bodies, infrastructure, and best management practices related to water resources (e.g., navigation, recreation). Population models may improve efficacy predictions for different chemical controls that target selected sensitive life stages, both by allowing a manager to test and rank various treatment techniques, and by optimizing the dosing application to achieve target goals. For such an approach to work, however, managers must obtain information on the population ecology specific to the target ANS to ensure that the most sensitive stages are present and thus affected by the treatment. In addition, the use of such models requires knowledge of the demographics and variability for the ANS species of concern. In many cases, field application of the control chemical would need to be repeated to establish long-term control or whenever densities of the susceptible target ANS life stages (e.g., *M. galloprovincialis* spawning events) increase to a predetermined project action level. The following conclusions can be drawn from the results:

(1) Larvae were much more sensitive to all three chemicals than adults and thus the chemical applications required may be lower for efficient control.
(2) Greater early life stage sensitivity to chemicals was observed prior to formation of a shell at the D-stage (i.e., <48 hr from fertilization).
(3) Adults are less susceptible to Cu and Cl_2 control than larvae due to a combination of greater resistance and valve closure.
(4) Sensitivity of adult *M. galloprovincialis* is higher in small mussels than in larger mussels.
(5) Pulse exposures with Cl_2 should be adequate for controlling larvae, but efficacy should be low for controlling adults.

The authors recommend development of control strategies that target the most susceptible ANS life stages (i.e., early life stages for *M. galloprovincialis*). By coupling toxicity information similar to that derived in this technical note with species population models, various control scenarios may be modeled to optimize management programs.

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