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LIFE HISTORY OF THE COPEPOD *Paramphiascella* sp. AFFECTED BY HYDROTHERMAL VENT EFFLUENTS

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Key words: Harpacticoid copepod, hydrothermal vents, Natural toxicity; Life cycle test, bioassay.

ABSTRACT

Toxicity of Hydrothermal vent (HV) effluents was tested by investigating the effect on growth and reproduction of the copepod *Paramphiascella* sp. which was collected and subsequently cultured from localities nearby a shallow marine HV at Kueishan Tao Island, Taiwan. Ontogenetic stages (nauplii, copepodids, and adults) were exposed to a range of concentrations of HV effluents in a static renewal culture system. In a first of two experiments we tested the survivorship of these in HV effluent dilutions from 1 to 50%. HV effluents significantly reduced the survivorship of the naupliar stages at concentrations >5% for *Paramphiascella* sp. ($p < 0.01$) and all nauplii died at concentrations of 25% and 50%. Copepodids were significantly letally affected at concentrations >5% ($p < 0.01$) and all died at 50% ($p < 0.01$). Developmental duration in *Paramphiascella* was showing a trend of developmental delay in both phases, in the naupliar and in the copepodid phase. Mortality showed a greater sensitivity to chemical exposure than development time. Among both traits were early developmental stages of *Paramphiascella* sp. more sensitive to HV effluents than advanced stages. We showed that *Paramphiascella* sp. was a useful test organism in the monitoring of life cycle as well as acute effects of HV effluents being present for millions of years and providing unique ecosystems. Mortality was a useful toxicological endpoint whereas developmental duration was not.

I. INTRODUCTION

There is an increasing need to monitor the environment due to pollution from anthropo-genic sources (e.g. effluent outfalls [71]), heavy metal emissions [48, 50, 51], EDCs [49], or natural sources, such as riverine sediment discharge [71] or effluents from hydrothermal vents [22].

Hydrothermal vent (HV) sites provide a habitat for a number of invertebrates auch as crabs, sea anemones, sea stars, crinoids, and sea fans [15, 36, 43] that have particular physiological and biochemical adaptations to their extreme habitat [47, 60, 72]. Kueishan Tao (or Turtle Island) is a Holocene volcanic island close to the NE Taiwan coast. HVs here are located about 50-60 miles away from HVs of the Okinawa Trough [45]. The HVs of Kueishan Tao are located at a tectonic junction of the fault system extension of Taiwan and the southern rifting end of the Okinawa Trough [43, 46, 74]. A group of about 50 HVs, at water depths between 20 to 70 m off the southeastern tip of Turtle Island, emits hydrothermal fluids and volcanic gases. These gases have a similar composition of low temperature fumaroles worldwide, with high H_2S and CO_2 but low HCl and SO_2 contents of a mantle source region that shows little crust contamina-tion [14, 75]. To what extend and how HV gases and effluents affect the biotic environment, particularly the plankton [23, 44], is largely unstudied as yet. Naturally occurring chemical stressors such as HV effluents have rarely been studied as yet. The reason being that their impact on human and environmental health has not been questioned as yet. Invertebrates play an increa-sing role in assessing impacts of environmental contaminants on marine ecosystems [21, 24, 28, 68]. Therefore, in recent years much emphasis was given to identify ecologically relevant and otherwise suitable toxicity models. Harpacticoid copepods provide a series of advantages which make them suitable candidates for such studies. Their position in marine food chains is prominent [19]. Furthermore, copepods play an important role in the transportation of aquatic pollutants within marine food webs. Knowledge has been increased for copepods, particularly in their evolution and zoogeography, ecology, behavior, and their biochemical and molecular responses following exposure to environmental stressors and chemicals [20, 25, 42]. Copepods are characterized by suitable traits for ecotoxicological testing, such as small size, distinctive life stages, short life-cycles, high fe-

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cundity. They usually have high densities, a wide distribution, and are reasonably easy to culture in the laboratory. Copepods are used in environmental genomics and ecotoxicology [67]. For several countries marine copepods are used in toxicology [30]. In the US there is a standardized microplate full life-cycle test available for the copepod *Amphiascus tenuiremis* [2]. Although several copepod species have been used in marine ecotoxicity testing and biomonitoring since the 1960s, the test organisms were primarily of adult stage [16, 29, 61]. Kusk and Wollenberger [52] proposed the four marine copepods, *Acartia tonsa*, *Amphiascus tenuiremis*, *Nitocra spinipes*, and *Tisbe battagliai* for the testing of endocrine-disrupting chemicals. A standardized, full life cycle bioassay with the estuarine copepod *A. tenuiremis* [2] has been used in some studies [1, 17, 70]. Multi-generation tests are important, especially for a holistic risk assessment of environmental pollutants. Harpacticoid copepods, such as *Tisbe* sp. and *Paramphiascella* sp. have several advantages, such as distinct sexual dimorphism, nauplius and copepodid stages, high fecundity, and short life cycles [26, 37] for another copepod species). Harpacticoid copepods show measurable toxic responses to an array of compounds and a range of temperatures and salinities [18].

The objectives of the present study was to compare the sensitivity of life cycle traits and acute toxicity of the harpacticoid copepod *Paramphiascella* sp. to HV effluents. The data will help to understand the concentration levels of HV effluents that may adversely affect natural populations of this species and its suitability for testing the toxicity of environmental samples.

II. MATERIAL AND METHODS

Adult copepods were collected on the 6th October 2007 from sediments about 500m away to the south from the main vent area of Kueishan Tao. There was no viable meiofauna and copepods in sediments directly exposed to the vents. Sediment retrieval was pursued as previously described [18]. Samples were transferred to the laboratory at the National Taiwan Ocean University and kept in oceanic autoclaved seawater (ASW) (34 psu) that had been taken from the NE coast of Taiwan about 5 km offshore away from the vent side and not been affected by HV effluents. *Paramphiascella* sp. have been cultured in the laboratory since August 2007. These copepods were maintained and cultured in the filtered (10 µm) natural sea-water obtained as described above (34 PSU salinity) at 24 ± 1°C and at a 12 h light to 12 h dark cycle. The ornamental fish food Tetra Min was provided as the sole food source ad libitum. Initially, approximately 1000 adult copepods were cultured in a 5 L quartz glas containers 24 ± 1°C. The copepods were used in two experiments that are explained below, both conducted at 28°C.

1. Exposure of Naupliar Stages to HV Effluents of Different Concentrations

HV effluents were tested for their effects on several developmental endpoints of the harpacticoid *Paramphiascella* sp. (for stage discrimination see Ferrari and Dahms [32]). The HV water for experimentation (several, subsequently taken 5L quartz glas bottle samples) was sampled directly from the bubbling chimney outlet or a HV site by a SCUBA diver (for chemical composition of HV effluent water, refer to Hwang unpubl.). Since concentrations higher than 50% always let to complete mortality within 24 h or faster, we tested the survivorship at a lower concentration range. This holded for concentrations of 1, 2.5, 5, 10, 25, and 50% of the original concentrations. Test solutions were renewed daily and the resulting nauplii and unhatched clutches were counted and removed under the stereomicroscope. Then 10 nauplii were transferred to multiwell plates containing 4 ml ASW, and about 100 ul of culture water that came along with the animals. Larvae at each of these stages were introduced to 7 HV effluent dilutions (control ASW, 1, 2.5, 5, 10, 25 and 50% HV effluent concentration). Each treatment of the 3 developmental stage groups, had 5 replicates, and each replicate contained 10 individuals. The larvae were transfered to fresh exposure media daily and maintained under the above-mentioned conditions for a total of 10 days. At each transfer, the numbers of surviving stages were counted.

2. Expt. I. Survival of Developmental Stages Exposed to HV Effluents

We tested the sensitivity of ontogenetic stages to HV effluents using nauplii, copepodids, and adults of *Paramphiascella* sp. The copepods were washed from their pre-culture containers and allowed to acclimate for 2d in ASW. Only healthy individuals were used in the experiment. The experiments consisted of the control (=ASW) and the following 6 HV effluent concentrations: 1, 2.5, 5, 10, 25, and 50 %. Each treatment had 5 replicates, and each replicate used 10 individuals in 20 ml solution. Experiments were run individually. No food was offered to the copepods but the exposure medium was renewed after 48 h. Finally, the survivors were counted, and the data were used to calculate survivorship.

3. Expt. II. Developmental Durations under HV Effluent Stress

The ontogeny of *Paramphiascella* (gamete to newly-settled juveniles) can be divided into 3 distinct periods (NI-NVI, CI-CVI, CVI to ovigerous females). Our experiment consisted of 3 bioassays. Here was each following the development of one group of developmental stages to the subsequent group of stages. Obtaining and handling organisms were similar as described by Dahms *et al.* [19].

Renewal of test solutions was carried out daily and Tetra Min suspension was added that could be consumed within a day (50% of the working volume). Developmental stages were checked daily under a stereomicroscope with a scattering light, and development stages and their time of development were recorded (*i.e.* from nauplii to copepodid, and from

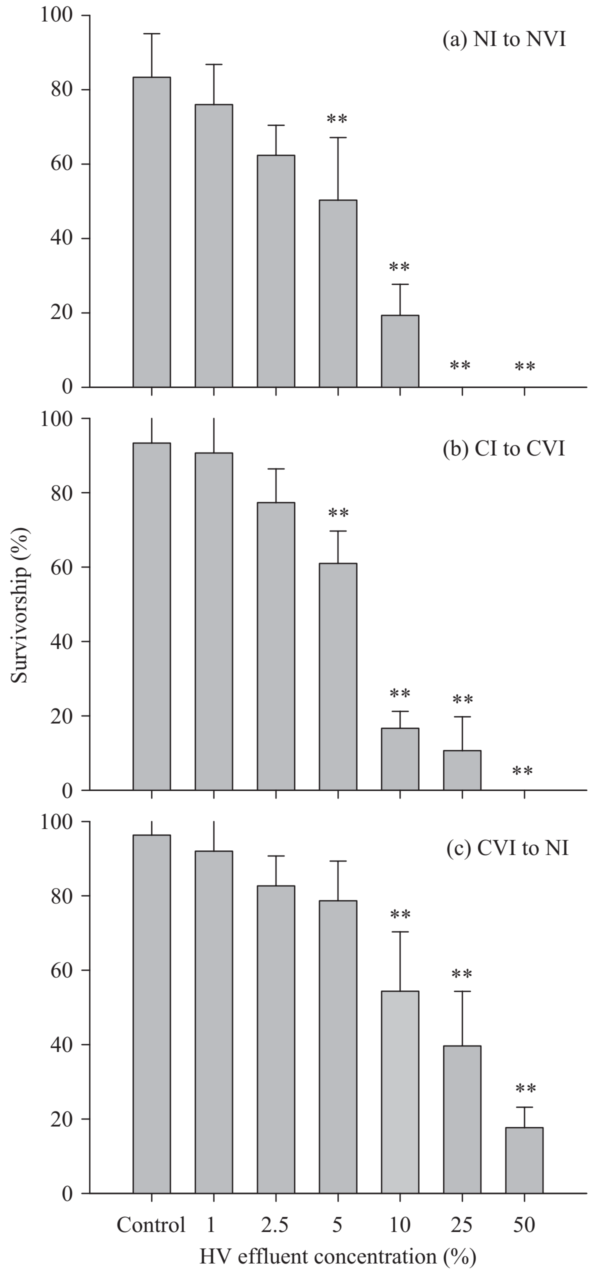

Fig. 1. ***Paramphiascella*** **sp. Effects of HV effluent concentrations on the survivorship of nauplii – NI to NVI, from CI to CVI, and from CVI to ovigerous females. The survivorship is plotted as mean ± SD of 5 replicate cultures of 10 individuals. * The mean difference is significant at the .05 level. ($P < 0.05$). ** The mean difference is significant at $p < 0.01$.**

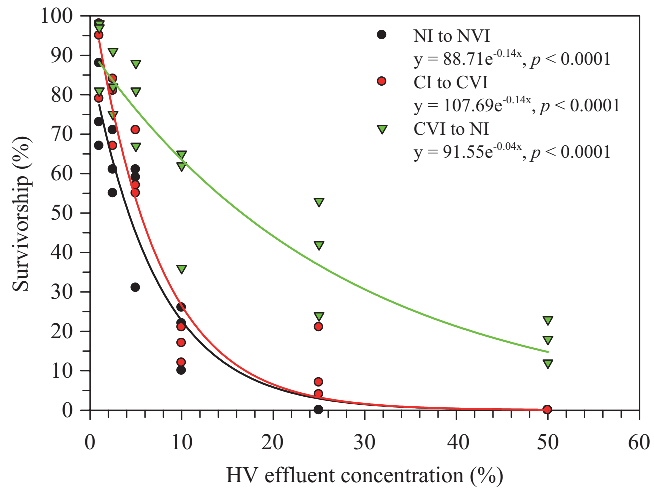

Fig. 2. Survivorship of ***Paramphiascella*** **sp. developmental groups (NI to NVI, from CI to CVI, and from CVI to ovigerous females) with increasing HV effluent concentrations showing significant differences between groups.**

nauplii to adults with egg sacs). Survival (%) was determined after all copepods were maturated. The maturation period in the control was on average 2 weeks but varied in the treatment groups.

4. Statistical Analysis

Duration of development data of survivorship were tested for normality by the Shapiro & Wilk Test [76]. The data were analyzed by non-parametric statistics since none of them met the normality assumption for parametric analysis. For this the values were transformed to ranks. Then parametric statistics were applied to ranks, as described in [76]. Data are presented as means ± standard deviation (S.D.). All statistical analyses were conducted using SPSS version 12.0 (SPSS Inc., Michigan Avenue, Chicago, Illinois, USA).

III. RESULTS

1. Expt I: Survival of Developmental Stages

HV effluents reduced the survivorship of the naupliar stages at concentrations >5% for *Paramphiascella* sp. significantly ($p < 0.01$). All nauplii died at concentrations of 25% and 50% (Fig. 1). Copepodids were significantly letally affected at concentrations >5% ($p < 0.01$) and no copepodid survived at 50% ($p < 0.01$). Surviviorship (versus mortality) turned out to be a useful toxicologically endpoint (Fig. 2).

2. Expt II: Developmental Durations

Representatives of neither the naupliar nor the copepodid phase in *Paramphiascella* showed a significant effect of developmental duration. However, a trend of developmental delay became apparent. Among the two concentration-dependently traits, showed mortality a greater sensitivity to chemical exposure than development time. In both traits were early developmental stages of *Paramphiascella* sp. more sensitive to HV effluents than were advanced stages (Fig. 3).

IV. DISCUSSION

Copepods have many attributes that make them an attractive group of organisms for toxicity testing in the aquatic realm [35, 52, 67].

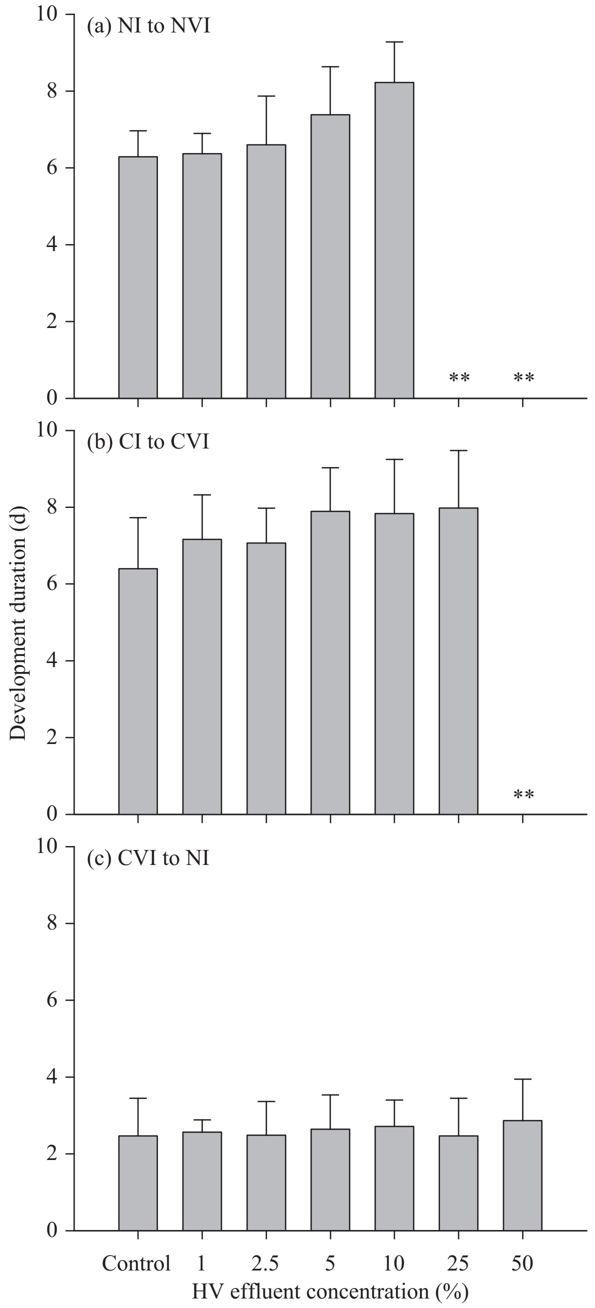

Fig. 3. *Paramphiascella* sp. Expt ID: Effects of HV effluent concentrations on developmental durations of NI to NVI, from CI to CVI, and from CVI to ovigerous females. The duration of development data are plotted as mean ± SD of 5 replicate treatments of 10 individuals. *The mean difference is significant at the .05 level. (*P* < 0.05). ** The mean difference is significant at *p* < 0.01.

A standardized test involving the estuarine copepod *A. tenuiremis* is used in the US [2]. However, as emphasized by Lee *et al*. [57] and also by Gourmelon and Ahtiainen [35], there is a need to develop and standardize more toxicity tests for meeting the regional environmental specificities and regulatory requirements. Four species of marine copepods, namely, *A. tonsa*, *N. spinipes*, *T. battagliai*, and *A. tenuiremis* have been identified as potential model species for EDCs [52]. In a recent overview, the OECD has highlighted *T. japonicus* as another species for toxicity testing risk assessment of EDCs [61].

A number of harpacticoid copepods were studied in ecotoxicology: *Amphiascus tenuiremis* [5, 11, 12, 70, 73]; *Tisbe battagliae* [4, 39, 40, 41]; and *Nitocra spinipes* [6, 8-10].

In the present study we studied the sensitivity of developmental stages of the copepods *Paramphiascella* sp. with respect to acute toxicity and the duration of developmental stages. These endpoints were easy to quantify, but they are not alltogether suitable for use in routine testing. Marcial *et al*. [59] for example reported that two endpoints, namely, the naupliar phase duration and development time for adults were most significantly affected by estrogenic compounds in the harpacticoid copepod *Tigriopus japonicus*. Lee *et al*. [58] demonstrated the usefulness of 2-generation studies in the ecotoxicological testing of this species. In the present study decreased survivorship was recorded in early developmental stages. The higher sensitivity at earlier stages is commonly the reason why embryos and larvae of invertebrates, such as mussels and oysters are widely used in toxicity tests [64, 65]. His *et al*. [38] found that among the early developmental stages of a species, embryos are usually more sensitive than larvae. The average EC_{50} for inhibition of molluscan embryogenesis was 39.8 $\mu g\ l^{-1}$ and the average LC_{50} for larval mortality was 86.5 $\mu g\ l^{-1}$. Besides, echinoderms [31] and polychaetes [64, 67] were used for ecotoxicological studies.

Tigriopus spp. and some copepod species have shown sensitivity to metals [52, 67]. Lee *et al*. [57] demonstrated the range and sensitivity of *T. japonicus* to various environmental toxicants and showed the effect of nine environmental contaminants on growth and developmental traits in a two-generation test [55].

Recently, Pedroso *et al*. [62, 63] have studied the toxicity of silver and its toxicological mechanism in the euryhaline pelagic copepod *A. tonsa*. They observed that Na^+ and K^+-ATPase plays a key role in silver toxicity in *A. tonsa*. The study of mechanistic aspects may be helpful for proper risk assessments of environmental toxicants. The molecular mechanisms of the toxicities of metals and EDCs in *T. japonicus* were studied using gene expression [55, 56, 67].

Toxicity results of chemicals are significantly affected by environmental variables [54]. Toxicities of certain chemicals are commonly affected by these variables. For instance, Kwok and Leung [53] observed that toxicities of Cu and TBT are significantly increased in *T. japonicus* when culture temperature was increased by 10°C. They also suggested that, at higher temperatures, animals undergo dormancy. Therefore, environmental variables and confounding factors have to be considered for the design of appropriate experimental procedures.

Since larval stages require feeding in order to complete their development, food was added. Organic material such as applied here, not only serves as food, but also provides binding sites for toxicants [3, 18]. When nominal toxicant concentrations are low, a large proportion of the toxicant might be bound to the algae, which are routinely used in the culture of invertebrate larvae, resulting in an underestimation of toxicity [13].

In addition to sensitivity to toxicants, environmental relevance and easiness of maintenance of an organism are also among the criteria for selecting species for routine bioassays. The copepod genus *Paramphiascella* is widely distributed along the worlds coasts [7]. Its ubiquitous distribution in marine waters worldwide let us assume that it is a common receiver of pollutants in coastal waters. At the experimental site in Kueishan Tao, this species seems to reproduce throughout the year, making it possible to obtain gametes for embryonic work in all seasons. Since this species is small (<800 um in length for females), many individuals can be cultivated in small dishes. We maintained populations of *Paramphiascella* sp. in 5-l aquaria by feeding the copepods with a suspension of TetraMin. There were approximately 50-75% ovigerous females at each sampling.

Copepods in general are particular in requiring less efford in laboratory space and in maintenance time. The harpacticoids *Nitocra psammophila* and *Amphiascus tenuiremis* have been used for environmental monitoring [33]. In a study by Geffard *et al*. [34] the harpacticoid *T. brevicornis* was used together with the oyster *Crassostrea gigas* and the sea urchin *Paracentrotus lividus* to assess sediment leachate toxicity in an assessment with 3 animals.

Life cycles and their respective endpoints provide suitable biomarkers in ecotoxicological monitoring [18, 27]. Although the duration of embryogenesis is short in sea urchins, *Tigriopus* and bivalves, the duration of their larval development is quite long (usually a month or longer), and the duration from settlement to maturation is even longer [66]. As a result from economic constraints imposed by chronic bioassays, most toxicity tests with bivalve larvae were conducted for not longer than 2 weeks [38]. This was without allowing the larvae to develop to eyed-veliger stages. A similar situation occured in bioassays using sea urchin larvae, where the larvae are generally maintained only for 3 to 4 days [13], being too short to complete the entire larval development.

As mentioned above is exposure duration another criterion to consider when applying appropriate tests. Under optimal laboratory conditions, the whole life-cycle of *Paramphiascella* sp. can be completed in about 15 d at 28°C, including less than 1 d for embryogenesis, 6 d for naupliar development, and 6 d for copepodid development.

The short life-cycle of *Paramphiascella* sp. offers, therefore, the potential for testing not only acute toxicity to various larval stages, but also sublethal growth and reproductive responses on all larval and juvenile stages within a relatively short period of time.

V. CONCLUSIONS AND PERSPECTIVES

Among the two different biological responses measured, mortality provides a gradual dose-response relationship and can be used as an endpoint in toxicity tests with this species. Developmental duration, however, does not correlate well with HV effluent concentrations at later stages in *Paramphiascella* sp. and *Tisbe* sp. thus is not a suitable endpoint for a bioassay for its toxicity. This study has shown that early developmental stages are much more sensitive to HV effluents than adults. Ecotoxicological testing should be performed in an integrated approach, involving conventional as well as advanced technologies that provide more relevant and realistic profiles of polluted environments.

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