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HYDROTHERMAL VENT EFFLUENTS AFFECT LIFE STAGES OF THE COPEPOD *TISBE* SP.

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Key words: copepod, hydrothermal vents, survivorship, natural pollution, life cycle.

ABSTRACT

We examined the environmental effects of various concentrations of hydrothermal vent (HV) effluents on growth, reproduction, and survivorship of the *Tisbe* sp. harpacticoid copepod that were retrieved from localities near the vent. Developmental stages (nauplii and copepodids) were exposed to various concentrations of HV effluents in a static renewal culture system. In the survivorship experiments, we tested 3 distinct developmental phases in HV effluent dilutions from 50% to 1%. The HV effluents considerably reduced the survivorship of the naupliar stages at concentrations of >1% ($P < 0.05$); all nauplii died at concentrations of 25% and 50%. The copepodids were considerably affected at concentrations of >1% in *Tisbe* sp. ($P < 0.05$), and no copepodid survived at 50% ($P < 0.01$). The adult females died at a 50% concentration in Tisbe sp. The developmental duration was not considerably affected in the naupliar or copepodid phases; however, it exhibited a trend of developmental delay. The naupliar development of *Tisbe* sp. was substantially delayed at a concentration of 10% ($P < 0.01$), whereas copepodids and adults only exhibited a trend of delayed development with increasing HV concentration. The endpoint mortality exhibited a greater sensitivity to chemical exposure than the endpoint development time. The early developmental stages of *Tisbe* sp. in both traits were more sensitive to HV effluents than advanced stages. Mortality was a useful toxicological endpoint compared that of developmental duration. We demonstrated that *Tisbe* sp. may be used in the monitoring of acute and life cycle effects of natural marine pollution caused by HV effluents.

I. INTRODUCTION

Hydrothermal vent (HV) sites provide a habitat for numerous crabs, sea anemones, sea stars, crinoids, and sea fans [19, 27, 36] that exhibit particular physiological and biochemical adaptations to extreme habitats [55, 69]. Gueishandao (or Turtle Island) is a Holocene volcanic island close to the northeastern coast of Taiwan with HVs that are located 60 miles from those of the Okinawa Trough [45]. The HVs of Gueishandao are located at a tectonic junction of the fault system extension of Taiwan and the southern rifting end of the Okinawa Trough [43, 46, 71]. A cluster of more than 50 HVs detectable by side scan sonar and echo sounder sensors at water depths between 10 m and 80 m off the southeastern tip of Gueishandao emit hydrothermal fluids and volcanic gases. The gases exhibit a similar composition of low temperature fumaroles worldwide, with high CO_2 and H_2S and low SO_2 and HCl contents of a mantle source region without considerable crust contamination [18, 72]. Previous studies have not examined the methods and the extent to which HV effluents and gases affect the biotic environment, particularly plankton [44]. Naturally occurring chemical stressors, such as HV effluents, are not a current research topic because their effect on people and environmental health was not a previous concern.

Invertebrates play a vital role in assessing the effects of environmental contaminants on marine ecosystems [63]. Therefore, considerable efforts were made recently to identify viable and ecologically relevant toxicity models. Harpacticoid copepods have various suitable advantages that make them candidates for such studies. Their position in marine food chains is prominent [23]. Copepods also play a vital role in the transportation of aquatic pollutants within marine food webs [67, 68]. The knowledge base of copepods has increased substantially recently, particularly regarding their evolution and zoogeography, ecology, behavior, and biochemical and molecular responses following exposure to environmental stressors and chemicals [24, 25, 42].

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Copepods are used in ecotoxicology and environmental genomics. Marine copepods have been used in toxicology [29] for several countries. A standardized microplate full life-cycle test is available in the United States for the copepod *Amphiascus tenuiremis* [2]. Although several copepod species were used in marine ecotoxicity testing and biomonitoring since the 1960s, the test organisms were mainly in the adult stage [20, 28, 37, 56, 62]. Kusk and Wollenberger [47] proposed 4 marine copepods (*Acartia tonsa*, *Amphiascus tenuiremis*, *Nitocra spinipes*, and *Tisbe battagliai*) for testing endocrine-disrupting chemicals. A standardized full life-cycle bioassay with the estuarine copepod *A. tenuiremis* [2] was used in various studies [1, 21, 66]. Multi-generation tests are crucial, especially for a holistic risk assessment of environmental pollutants. Harpacticoid copepods, such as *Tisbe* sp., have several advantages, such as distinct sexual dimorphism, nauplius and copepodid stages, high fecundity, and a short life-cycle - see [26] (for another *Tisbe* species). Harpacticoid copepods exhibit measurable toxic responses to an array of compounds and various temperatures and salinities.

We compared the sensitivity of life-cycle traits of *Tisbe* sp. to HV effluents and examined the acute toxicities and life-cycle parameters of HV effluents. The data facilitated the understanding of the concentration levels of HV effluents that may adversely affect the wild population of this species and its suitability for testing the toxicity of environmental samples.

II. MATERIAL AND METHODS

1. Copepod, Sediment Collection, and Handling

Adult copepods were collected on October 6, 2007 from sediments approximately 500 m south of the main vent area of Gueishandao because the sediments that were directly exposed to the vents were void of meiofauna. Sediment retrieval was performed as previously described [22]. The samples were transferred to the laboratory at the National Taiwan Ocean University and stored in pristine oceanic autoclaved seawater (ASW) (34 psu) obtained from the northeastern coast of Taiwan, approximately 5 km offshore from the vent site. The *Tisbe* sp. were cultured in a laboratory since August 2007. These copepods were maintained and cultured in filtered (10 μm) natural seawater (salinity 34 PSU) at 24 ± 1°C in a 12 h light to 12 h dark cycle. Ornamental Tetra Min fish food was provided. Initially, approximately 1000 adult copepods were cultured in a 5 L glass container at 24 ± 1°C. The copepods were used in 2 experiments conducted at 28°C.

2. Exposure of Naupliar Stages to HV Effluents of Various Concentrations

The HV effluents were tested for their effects on several attributes of copepod development. Because concentrations higher than 50% consistently resulted in mortality within 24 h or longer exposure time, we conducted testing at a lower concentration range; that is, 1%, 2.5%, 5%, 10%, 25%, and 50% of the original concentrations. The test solutions were replaced daily, and the resulting nauplii and unhatched clutches were counted and removed under a stereomicroscope. Ten nauplii were transferred to multiwell plates containing 4 mL of ASW and approximately 100 μL of culture water that was obtained with the animals. The larvae at each stage were introduced to 7 HV effluent dilutions (control ASW, 1%, 2.5%, 5%, 10%, 25%, and 50% HV effluent concentrations, respectively). Each treatment of the 3 developmental stage groups had 5 replicates, and each replicate contained 10 individuals. The larvae were transferred to fresh exposure media daily and cultured under the stated conditions for 10 d. The number of surviving larvae and new settlers were counted at each transfer. Powdered ornamental fish food (Tetra Min) was used as the staple food and provided daily ad libitum.

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

The sensitivity of the developmental stages to HV effluent stressors was tested using nauplii, copepodids, and adults of *Tisbe* sp. The copepods were washed from the sediments and allowed to acclimate for 2 d in ASW. Only healthy individuals were used in the experiment. The experiment consisted of the control (=ASW) and 6 HV effluent concentrations of 1%, 2.5%, 5%, 10%, 25%, and 50%, respectively. Each treatment had 5 replicates, and each replicate had 10 individuals in 20 mL solution. The experiments were conducted individually. No food was provided to the copepods; however, the exposure medium was renewed after 48 h. At the end of the treatment, the survivors were counted, and the data were used to calculate survivorship.

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

The early development of *Tisbe* sp. was divided into 3 distinct periods (i.e., NI-NVI, CI-CVI, and CVI to ovigerous females). The experiment consisted of 3 bioassays, each following the development of 1 group of developmental stages to the subsequent group of stages. The acquisition and handling of the organisms were conducted in a similar manner to that used by Dahms *et al*. [23].

The test solutions were renewed daily (50% of the working volume), and food suspension for consumption within a day was added. The developmental stages were observed daily under a stereomicroscope with scattering light and recorded to calculate the time of development (i.e., from nauplii to copepodid and from nauplii to adults with egg sacs). The survivorship (%) was determined after the maturation of all copepods. The maturation period in the control was an average of 14 d; however, it varied in the treatment groups.

5. Statistical Analysis

Data regarding the survivorship and duration of development were assessed for normality using the Shapiro-Wilk test. The data were analyzed using non-parametric statistics because the data did not meet the normality assumption for

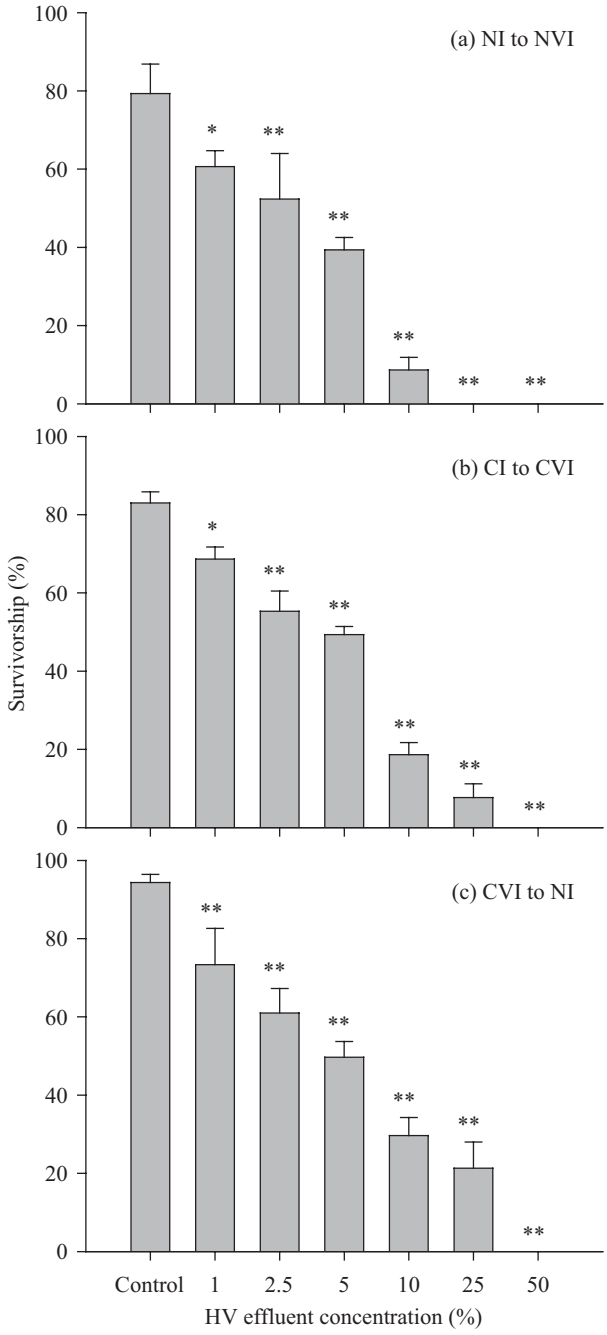

Fig. 1. Effects of HV effluent concentrations on the survivorship of nauplii: from NI to NVI, from CI to CVI, and from CVI to the ovigerous females of *Tisbe* sp. The survivorship is plotted as mean ± SD of 5 replicate cultures of 10 individuals. * The mean difference is significant at the 0.05 level.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

parametric analysis. This was performed by transforming the values to ranks, and subsequently, applying parametric statistics to the ranks, as described in [73]. Data were presented as means ± standard deviation (SD). All statistical analyses were conducted using SPSS version 13.0 (SPSS Inc., Michigan Avenue, Chicago, Illinois, USA).

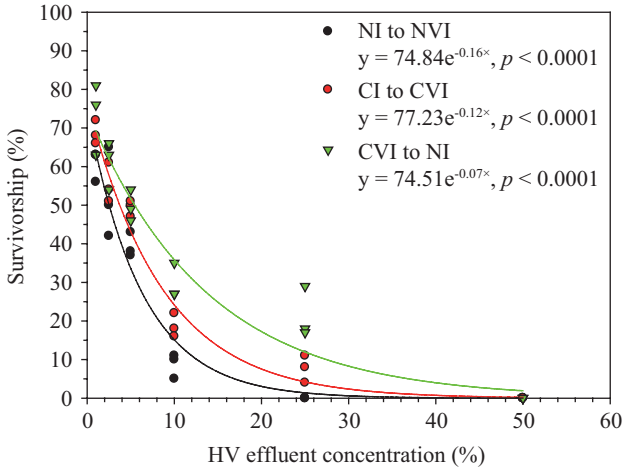

Fig. 2. Survivorship of *Tisbe* sp. developmental groups (from NI to NVI, from CI to CVI, and from CVI to ovigerous females) with increasing HV effluent concentrations exhibiting considerable differences between groups.

III. RESULTS

1. Expt I: Survival of Developmental Stages

The HV effluents considerably reduced the survivorship of the naupliar stages at concentrations of >1% for *Tisbe* sp. ($P < 0.05$), and all nauplii died at concentrations of 25% and 50% (Fig. 1). The copepodids were lethally affected at concentrations of >1% and no copepodid survived at 50% ($P < 0.01$). All adult females died at a 50% concentration in *Tisbe* sp. Mortality provided, thus, a useful toxicological endpoint (Fig. 2).

The survivorship in the 3 stages of *Tisbe* sp. exhibited a substantially negative trend with increasing concentrations of HV effluents (Fig. 2). Mature individuals exhibited an optimal ability to tolerate higher concentrations of HVs. Survivorship was zero for stages NI to NVI and CI to CVI at HV effluent concentrations of 50%. The trend curves of survivorship indicated a faster decrease at stages NI to NVI than those of stages CI to CVI and CVI to NI. The trend curves showed that the LC_{50} (lethal concentration, 50%) of adult individuals (CVI to NI) had an HV effluent concentration of approximately 6%. The LC_{50} for stages NI to NVI and CI to CVI were HV effluent concentrations of approximately 2.5% and 4%, respectively.

2. Expt II: Developmental Durations

The developmental duration was not considerably affected in the naupliar phase and copepodid phase; however, it exhibited a trend of developmental delay (Fig. 3). In the *Tisbe* sp. the naupliar development was substantially delayed at a concentration of 10% ($P < 0.01$), whereas copepodids and adults exhibited a trend of delayed development with increasing HV concentration.

Mortality exhibited a greater sensitivity to chemical

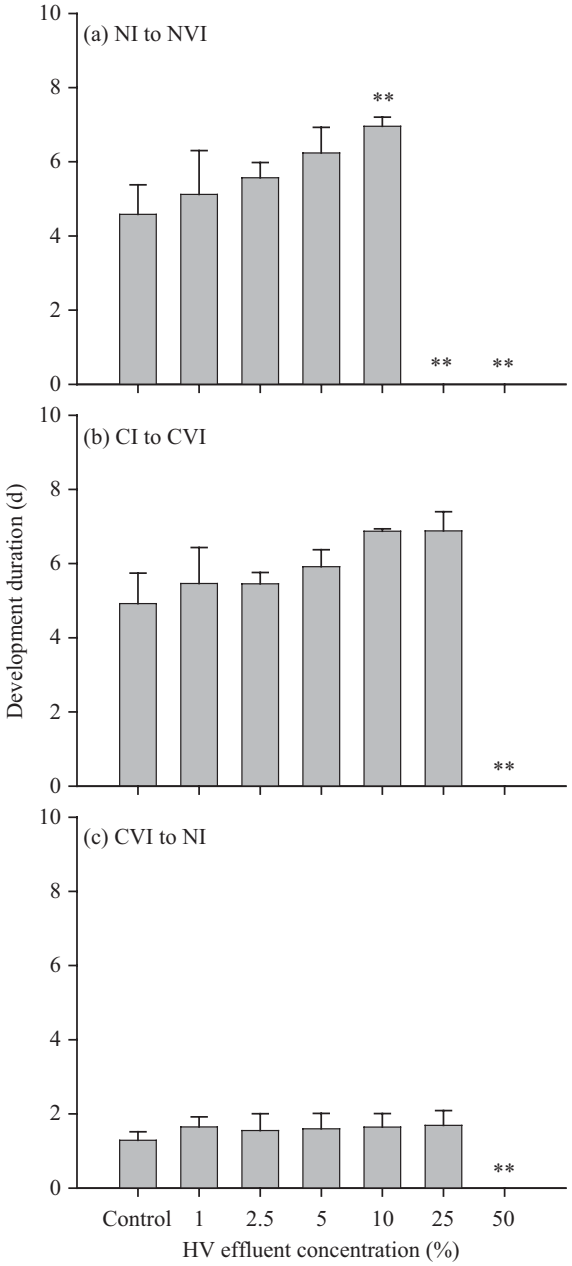

Fig. 3. Expt ID: Effects of HV effluent concentrations on the developmental durations of NI to NVI, from CI to CVI, and from CVI to ovigerous females of *Tisbe* sp. The duration of development data are plotted as mean ± SD of 5 replicate treatments of 10 individuals. ** The mean difference is significant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

exposure than development time did. For both traits, the early developmental stages of *Tisbe* sp. were more sensitive to HV effluents than the advanced stages were. The effect was concentration-dependent.

IV. DISCUSSION

We explored the sensitivity and duration of the developmental stages of the copepod *Tisbe* sp. to acute toxicity. These endpoints were easy to quantify; however, they were unsuitable for use in routine testing. Marcial *et al*. [54] reported that 2 endpoints (the naupliar phase duration and development time for adults) were considerably affected by estrogenic compounds in the harpacticoid copepod *Tigriopus japonicus*.

Increased mortality was observed in the early developmental stages of both copepod species; therefore, the embryos and larvae of invertebrates, such as mussels and oysters, were used in the toxicity tests partially because of their high sensitivity [59, 60]. His *et al*. [38] indicated that, among the early developmental stages of a species, embryos are usually more sensitive than larvae. The average EC_{50} for inhibition of molluskan embryogenesis was 39.8 µg l^{-1} and the average LC_{50} for larval mortality was 86.5 µg l^{-1} in echinoderms [31] and even higher in polychaetes [59, 64].

Species of the copepod *Tigriopus* spp. and other copepod species exhibited sensitivity to metals [47, 62]. Lee *et al*. [51] demonstrated the range and sensitivity of *T. japonicus* to various environmental toxicants and the effect of 9 environmental contaminants on the growth and developmental traits in a two-generation test [52].

Pedroso *et al*. [57, 58] examined the toxicity of silver and its mechanism in the euryhaline pelagic copepod *A. tonsa*. They observed that Na^+ and K^+-ATPase plays a vital role in the silver toxicity of *A. tonsa*. A study of the mechanisms involved may be helpful for a proper risk assessments of environmental toxicants. Molecular mechanisms of the toxicities of metals and EDCs in *T. japonicus* were studied using gene expression [52, 53, 62].

The toxicity results of chemicals are considerably affected by environmental variables [49]. The toxicities of numerous chemicals are affected by these variables. For example, Kwok and Leung [48] observed that the toxicities of Cu and TBT substantially increased in *T. japonicus* when the culture temperature was increased by 10°C. They also suggested that animals may undergo dormancy at higher temperatures. Therefore, environmental variables and confounding factors must be considered in the design of appropriate experimental procedures.

Food was added because species in the larval stages require feeding to complete their development. Organic material, such as dried ornamental fish food, serves as food and provides binding sites for toxicants [5, 22]. When nominal toxicant concentrations are low, a large proportion of the toxicant may bind to the algae, which are routinely used in the cultures of invertebrate larvae and result in an underestimation of the toxic effect [17].

In addition to the sensitivity to toxicants, the environmental relevance and ease of maintenance of an organism are among the criteria for selecting species for routine bioassays. The taxon Tisbe is extensively studied. Its species are easy to collect, store, and rear in the laboratory [26]. Consequently, they are suitable organisms for various morphological, ecological, and genetic studies. This is particularly accurate for

Tisbe battagliai [39, 40], but also applies to other species of *Tisbe*. We were aware of the optimal rearing techniques for several representatives of *Tisbe* [3, 4], including diet and salinity requirements [65], demographic characteristics [9], karyology [50], ecology and population dynamics in the field [30], postembryonic development [32], and the ecotoxicology of *T. battagliai* [6, 39, 40]. In particular, both copepod species require less laboratory space and maintenance time. The harpacticoids, *Amphiascus tenuiremis* and *Nitocra psammophila*, were used for environmental monitoring [33]. Geffard *et al*. [34] used the harpacticoid *T. brevicornis* with the oyster *Crassostrea gigas* and the sea urchin *Paracentrotus lividus* to assess the sediment leachate toxicity in a three-tier assessment.

Although the duration of embryogenesis is short for sea urchins and bivalves, the duration of their larval development is long (usually a month or longer), and the duration from settlement to maturation is longer [61]. Most toxicity tests with bivalve larvae were conducted for no longer than 2 wk [38], and did not allow the larvae to develop to the eyed-veliger stage because of economic constraints imposed by chronic bioassays. A similar situation occurs in bioassays that use sea urchin larvae as testing organisms; the larvae are generally maintained for only 3 to 4 d [17], which is insufficient to complete larval development.

Exposure duration is another criterion to consider when applying appropriate tests. Under optimal laboratory conditions, the whole life-cycle of *Tisbe* sp. can be completed in 12.5 d at 28°C, including less than 1 d for embryogenesis, 5 d for naupliar development, and 6 d for copepodid development. Therefore, the comparatively short life cycle of *Tisbe* sp. offers the potential for testing acute toxicity at various larval stages, and testing the sublethal growth and reproductive responses in the whole larval and juvenile stages within a relatively short period.

Copepods have several attributes that make them suitable organisms for toxicity testing in the aquatic field [35, 47, 62]. A standardized test using the *A. tenuiremis* estuarine copepod is used in the United States [2]. However, Lee *et al*. [35, 51] indicated that more toxicity tests must be developed and standardized for meeting regional environmental specificities and regulatory requirements. Four species of marine copepods (i.e., *A. tonsa*, *N. spinipes*, *T. battagliai*, and *A. tenuiremis*) were identified as potential model species for EDCs [47]. In a recent study, the OECD indicated that *T. japonicus* is another species suitable for toxicity testing and risk assessment of EDCs [56]. Numerous harpacticoid copepods were studied in ecotoxicology, including *Amphiascus tenuiremis* [7, 15, 16, 66, 70], *Tisbe battagliae* [6, 39-41], and *Nitocra spinipes* [8, 11-14].

V. CONCLUSION AND PERSPECTIVES

In summary, this study demonstrated that the early developmental stages are considerably more sensitive to HV effluents than the adults of the species. Between the 2 distinct measured biological responses, mortality provided a gradual dose-response relationship that can be used as an endpoint in toxicity tests with this species. However, the developmental duration did not effectively correlate with HV effluent concentrations at later stages in *Tisbe* sp.; therefore, it is an unsuitable endpoint for a bioassay because of its toxicity. In general, copepods exhibited favorable characteristics for ecotoxicological testing, such as short life-cycles, high fecundity, small size, and distinctive life stages. They usually have high densities, a wide distribution, and are reasonably easy to culture in a laboratory. Ecotoxicological testing must be performed in an integrated approach that involves conventional and advanced technologies to provide more relevant and realistic profiles of polluted environments.

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