A COMPARISON OF METHODS FOR SAMPLING SPLASHPOOL COPEPODA

JAMES J. POWLIK

SARSIA

POWLIK, JAMES J. 1998 08 28. A comparison of methods for sampling splashpool copepoda. – *Sarsia* 83:219-223. Bergen. ISSN 0036-4827.

Collection methods for micro- and meiofauna within high shore splashpools are compared using four simple, hand-held devices, with the supralittoral harpacticoid copepod *Tigriopus californicus* as a representative organism. For the collection of all *T. californicus* life-history stages, over a range of potential densities and with a minimum of specimen damage, a 30 ml graduated pipette is demonstrably more efficient than bottles, preparation dishes, or small nets. Collection of nauplii and gravid females, which typically subsist closer to the substratum and in pits or crevices in the bedrock, was particularly better using the pipette, which generates a rapid suction, produces less overall disturbance than larger devices, and can be used over a broad range of pool sizes without modification. For analysis, pipettes provide a smaller, more concentrated sample, reducing processing time and the potential for handling error.

James J. Powlik, National Science Foundation, 4201 Wilson Blvd., Room 835, Arlington, Virginia 22230, USA.

KEYWORDS: Sampling; *Tigriopus*; supralittoral; methods; splashpool.

'When we deliberate it is about means and not ends.'
– ARISTOTLE (4TH C. B.C.)

INTRODUCTION

Isolated or semi-isolated natural pools have been useful as microcosms for in situ study of aquatic ecology. Recent studies include BROWN 1991; MAHON & MAHON 1994; TABLADO & al. 1994; DYBDAHL 1995; HUNT & al. 1995; and METAXAS & SCHEIBLING 1996; see also the comprehensive review of METAXAS & SCHEIBLING 1993 and citations therein. However, such studies depend on accurate methods for sampling micro- and meiofauna. Methods for open water sampling, such as net and pump systems, may not be applicable on a more restricted spatial scale. The shallow depth of most pools precludes the use of more traditional sieves, nets or siphons, which cannot be properly submerged or maneuvered. Nevertheless, the same design considerations (the size, weight and stability of the device; mesh size; intake diameter and velocity) and deployment considerations (tow rate, wire angle, depth, and volume of the sample) may be applicable (see, e.g. POWLIK & al. 1991). The best choice of sampling device may be affected by the behavior of the organism, including its escape potential, position in the water column and sensitivity to light and pressure cues – reactions which, in turn, can vary substantively with the organism's life-history stage, ambient weather or water conditions, and the fishing skill of the researcher.

Sampling methods for tide pool or splashpool studies have not heretofore been sufficiently tested and compared (but see CHULLASORN & al. 1993). Within the supralittoral habitat, *Tigriopus* copepods present a convenient subject for evaluating sampler design. Though not large compared to some copepod species, *T. californicus* (1200-1500 µm in length at the fully-grown C-VI stage) are easy to observe in culture by their orange-red coloration and erratic swimming style and are often found abundantly in situ, to the near complete exclusion of other species. The species exhibits six naupliar and six copepodite stages from the egg through the mature adult. It is found on the shores of nearly every ocean, and can flourish in its shallow (typically less than 10 cm), low volume (1 to 1000 liters) natural microcosms to densities of over 200 000 individuals · l^{-1} (POWLIK & LEWIS 1996; POWLIK & al. 1997).

The devices used to sample or enumerate splashpool organisms have ranged from sawed-open plastic bottles to finger bowls and gas-powered pumps, with most of these devices admittedly jerry-rigged simply and economically with little claim to empirical sampling. As examples, FRASER (1936) and MONK (1941) sampled *Tigriopus* pools by siphoning water through a net or bailing the pool of all its water, an effective collection procedure, but one which may be too disruptive of the habitat for mensurative investigation. BURTON & FELDMAN (1981) collected *Tigriopus californicus* using plastic bottles or, at lower population densities, a fine mesh. EGLOFF (1966) used a 0.5 l bowl and HARRIS (1973)

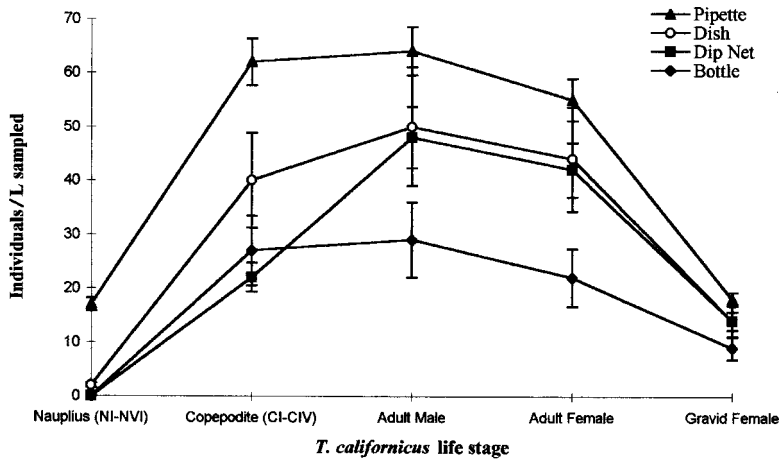

Fig. 1. Density of *Tigriopus californicus* from the random deployment of four samplers in nine *Tigriopus californicus* splashpools in Barkley Sound, British Columbia. Triplicate samples from each pool were combined to derive the mean population density of each pool, then plotted as mean ± s.e. for the life-history stages indicated. See text for specifications of each sampler.

drew 100 ml samples through a 63 µm sieve. Dethier (1980) estimated population numbers from the number of individuals passing over a small Secchi disk, a method that permits enumeration but not specimen identification.

With the above in mind, a comparative trial was devised to test the sampling ability of a number of these informal but commonly-used pool samplers, using ambient populations of *Tigriopus californicus* as a gauge. The efficiency of four sampling devices was determined by comparing the number of individuals of each life-history stage collected, compared to the number of specimens extant in enumerated laboratory populations or estimated natural populations.

MATERIAL AND METHODS

Four sampling devices were selected according to citations of their use in published studies. Each device is guided by hand and used to collect a discrete water sample with a minimum of disturbance to the pool environment: 1) a 500 ml Nalgene bottle with mouth opening of 6 cm; 2) a dip net fashioned from 200 µm mesh stretched over a kitchen strainer (mouth opening of 20 cm); 3) a 450 ml preparation dish with a 12 cm mouth opening; and 4) a 30 ml graduated pipette with an intake port of 1 cm diameter.

Among field sites in Barkley Sound, British Columbia (see Powlik & Lewis 1996; Powlik & al. 1997), each device was deployed in three randomly-selected positions within each of 9 pools containing populations of *Tigriopus californicus*. The order of sampler deployment was also varied randomly between pools to minimize error associated with pool disturbance. Copepods in each sample were categorized as nauplii (stages N-I to N-VI), copepodites (stages C-I to C-IV), adult males and adult females (the sexually dimorphic C-V and C-VI stages), and gravid females, then each category was enumerated. Attached to gravid females until deposition, *T californicus* eggs were not enumerated.

Heterogeneous distributions of the copepod are common in situ, hence field population numbers cannot be absolutely known without pumping the pool dry and scouring the exposed substratum. To simulate such an estimate, a controlled comparison was produced in the laboratory by filling an 8 liter (20 cm × 50 cm × 8 cm) glass dish with a culture of *T. californicus*. Between each use of each sampler, all collected copepods were categorized to approximate life stage, enumerated, then returned to the dish by thoroughly rinsing the sampler with clean sea water and decanting the excess wash from the original culture volume. For each sampler, a total of 9 trials were conducted over a period of 12 hours, hence potential error associated with life stage molts was minimized. All counts were adjusted to reflect the actual volume of water collected by each sampler, per Powlik & Lewis (1996). After all trials were completed, all copepods in the dish rinsed out, categorized, and enumerated.

Since a normal distribution and homogeneity of variance could not be assumed for the copepod populations (especially for in situ populations), sampler performance was compared using a nonparametric randomized block analysis of variance (Friedman's Test, sensu Zar 1984) at $\alpha = 0.05$.

RESULTS

The number of each category of *Tigriopus californicus* collected from natural splashpools by the four sampling devices is summarized in Fig. 1. The graduated pipette demonstrates a greater collection of all categories, but this difference was statistically significant (Friedman's Test, $\alpha = 0.05$, $0.01 < P < 0.05$, d.f. = 26) only for

nauplii, copepodites and gravid females. These categories of *T. californicus*' life-history stages are typically found in greater numbers along the substratum and are more easily accessed by the pipette than samplers with a larger intake. For adult males and females, nets, dishes, and pipettes collected approximately equal numbers of copepods from splashpools. Overall, bottles collected the fewest number of individuals, a result of a small intake combined with an inability to maneuver the device close to the pool bottom.

Table 1 also shows the pipette collecting significantly greater numbers (Friedman's Test, $\alpha = 0.05$, $0.01 < P < 0.05$, d.f. = 8) of all life-history categories from culture populations, with the exception of adult males and females. Adults, which more commonly occupy the water column, were sampled about as well by all samplers. The net was significantly less effective at sampling younger life stages, probably because they passed through the mesh (see Powlik & al. 1997).

DISCUSSION

The most prominent obstacle to using pools as microcosms in ecology and evolution is the accurate and representative sampling of all life-history stages. Credible estimates allow the derivation of birth and death rates, clutch size, egg viability, time of first hatching, and larval survival under natural conditions. *Tigriopus californicus* utilize the full extent of the water depth available in their pools, with dense populations often coloring the water orange with 'clouds' or 'swarms' of swimming individuals. The review of Hicks & Coull (1983) and the work of Bell & al. (1988) also describe mature females of other copepod species to favor the substratum, while males and immature females may utilize the available water column to enhance the opportunity for precopulatory encounters. Feeding of all life-history stages occurs along the substratum as the organism browses surfaces for bacteria or microalgae, however

Table 1. Comparison of *Tigriopus californicus* culture population to numbers collected by each sampler. 'Count' denotes actual numbers in 8 liters or average number sampled in $n = 9$ trials. '% Collected' denotes the proportion of each life-history stage collected by each sampler, based on mean values rounded to the nearest whole individual. Boxed areas denote categories of statistical significance (Friedman's Test) at $\alpha = 0.05$.

	Actual	Pipette	Dish	Net	Bottle
Nauplii					
Count	300	41	6	4	0
Range	296 - 305	22 - 76	0 - 16	0 - 6	0 - 0
s.e.	0.75	5.89	1.57	0.67	0.00
% Collected	(100)	13.67	2.00	1.33	0
Copepodites (CI-CIV)					
Count	450	71	60	51	6
Range	—	55 - 93	53 - 69	25 - 123	0 - 8
s.e.	0.00	5.11	1.88	11.79	1.06
% Collected	(100)	15.78	13.33	11.33	1.33
Adult males (CV-CVI)					
Count	274	51	56	44	13
Range	271 - 278	35 - 81	19 - 71	12 - 61	0 - 19
s.e	1.87	6.01	4.93	5.96	2.23
% Collected	(100)	18.61	20.44	16.06	4.74
Adult females (CV-CVI)					
Count	261	41	43	32	10
Range	257 - 264	18 - 56	12 - 51	4 - 39	0 - 13
s.e.	0.92	4.51	4.78	3.66	1.50
% Collected	(100)	15.71	16.48	12.26	3.83
Gravid females					
Count	78	10	4	6	2
Range	—	0 - 16	0 - 9	2 - 8	0 - 3
s.e.	0.00	1.79	0.78	0.65	0.33
% Collected	(100)	12.82	5.13	7.69	2.56
Overall					
Count	1363	214	169	137	31
Range	1351 - 1386	189 - 322	84 - 216	43 - 237	0 - 43
s.e.	3.78	14.27	13.02	22.07	4.55
% Collected	(100)	15.70	12.40	10.05	2.27

this does not preclude incidental filtering of materials in solution or the uptake of dissolved organic material across the cuticle (e.g., HARRIS 1973). Consideration of the organism's ontogeny is not incidental.

Despite its relatively small, discrete habitat, representative sampling of *Tigriopus*' various life stages cannot be assumed, as its size, shape, behavior, nutritional preferences, and location in the water column will each vary. As an adult, the organism exhibits a dive-and-cling swimming behavior (EGLOFF 1966; VITTOR 1971), and an escape response initiated by shadows or microscale changes in hydrostatic pressure. Adults may employ the full depth of water available to them, or may browse the bottom of pools – this is especially true of the larger, heavier egg-bearing females.

The magnitude of any escape response will also vary with the size of the sampler used, the speed and care of its towing through the water, and its detection by the organism, which is related to the sampler's appearance and design, the time of day and ambient weather conditions. During several nighttime collections (unpublished data), a flashlight beam trained directly on a swarm of *Tigriopus* in the water column of their pool produced neither a positive nor a negative phototaxis, possibly indicating that the intensity of the light was not sufficient to elicit phototaxis. It has not yet been determined whether the organism may respond similarly to changes in pressure or light from the non-visible spectra. Since any disturbance will be magnified accordingly in a restricted volume, particular care should be taken to avoid casting shadows and undue agitation of the pool water prior to collection.

Given the heterogeneity or patchiness of *Tigriopus californicus* populations, a random or stratified-random method for selecting sample locations is the most desirable. Although temporary stratification of splashpools may occur under static conditions, this condition is easily disrupted by wind or wave action, and it has not been determined whether the organism preferentially associates with microscale thermoclines or haloclines within pools. (This would be doubtful, given the organism's demonstrated tolerance to temperature, salinity and pressure under laboratory conditions.)

In application, pipettes offer unique advantages, including: 1) relatively less disturbance of the habitat and virtually no specimen damage; 2) a controlled, high-speed collection of organisms, owing to the smaller intake; and 3) a comparatively less voluminous (dilute) sample, which both reduces the amount of processing time and the potential for error from sample splitting or repeated transfer. The disadvantage of pipettes are applicable to all the samplers tested: The smaller intake and relatively smaller volume of water sampled makes the device more susceptible to microscale patches of organisms, although this can be somewhat remedied by sampling design. In sampling closer to the bottom, there is the risk of under-sampling copepods nearer the surface or otherwise suspended in the water column. This latter point can be addressed by sampling at several levels throughout the pool's depth, and may not be an issue in some applications.

Although *T californicus* exhibit a quick-start potential, all stages appear to fatigue quickly and soon settle-out when placed in deeper volumes of water (isolated pools, in the form of flooded crevices, may reach 30 to 60 cm in depth) and this response has been observed in laboratory aquaria. All samplers collected higher proportions of nauplii and copepodites from the culture dish, but this was likely an artifact of 1) a lack of pore spaces and pits within which these life stages can avoid sampling; and 2) the comparatively greater suspension of all life-history stages in the culture dish, a result of more frequent disturbance and replenishment of the water.

Bottles were least effective at sampling, a combined result of their smaller intake (relative to nets or dishes) and inability to sample close to the bottom (relative to pipettes), while nets and preparation dishes were somewhat intermediary in their utility. Both nets and dishes offer the advantages of sampling a larger volume of water, making them less susceptible to small-scale patches.

Dishes offer comparatively less entanglement and damage of specimens than nets, but the resistance of water 'piled up' before the dish may be unacceptably large unless the angle of attack is lessened (to ca. 45° to 60°, rather than perpendicular). Given the size of the organism relative to all samplers and the size of the pool, the speed at which samplers are dragged through the pool must also be considered, as the pressure wave advancing before a rapidly-dragged sampler is apt to displace individuals of all life-history stages, regardless of their swimming ability. From POWLIK & al. (1997), *T californicus* nauplii do not exhibit directed swimming behavior prior to the N-III stage, hence for younger individuals avoidance of the sampler is less an 'escape response' than displacement by the hydrodynamic disturbance of the sampler itself. Here again, pipettes offer a distinct advantage.

Nets permit the largest volume of water to be sampled – and are probably preferred for sampling from the water column in deeper pools – but typically cannot access the substratum sufficiently. Conversely, nets also *require* the greatest amount of water to be used properly, while pipettes can be used equally well in water deposits ranging from a few milliliters to hundreds of liters, provided that pool depth does not require submerging the suction

bulb. Nets also require more maintenance, and mesh size must be selected as appropriate to the organism and life stage of interest. Discounting inequalities in the dipping/dragging of the device (and unlike dishes, pipettes, or bottles), the capacity and efficiency of a small net also cannot be assumed to be truly consistent each time it is used in such restricted confines. The actual volume of water sampled by a net is imprecisely known, which can produce an additional source of variation or error. Hence, for small volume pools, a pipette may be the only consistent sampling method over all potential sites.

Finally, the density of field populations is better presented as individuals per unit pool volume, rather than per unit of volume sampled. Samples recorded only as individuals · ml^{-1} or · l^{-1} may erroneously suggest a bloom or decline in copepod abundance, when what is actually indicated is evaporation or dilution of the pool itself. Since pool volume can change over as little as a few hours. Even pumping all the water from a given pool (to derive an accurate estimate of pool volume) may not sufficiently collect all individuals from crevices in the incised bedrock, and may even reduce estimates from those obtained from pools sampled while filled.

ACKNOWLEDGMENTS

The author wishes to thank the *Auu ay aht* Indian Band for providing access to the collection sites and J.D.G. Boom for providing logistic support. For Úna.

REFERENCES

Aristotle, 4th c. B.C. *Nicomachean Ethics* 3.3 tr. J.A.K. Thomson.

Bell, S.S., G.R.F. Hicks & K. Walters 1988. Active swimming in meiobenthic copepods of seagrass beds: Geographic comparisons of abundances and reproductive characteristics. – *Marine Biology (Berlin)* 98:351-358.

Burton, R.S. & M.W. Feldman 1981. Population genetics of *Tigriopus californicus* II: Differentiation among neighboring populations. – *Evolution* 35:1192-1205.

Brown, A.F. 1991. Outbreeding depression as a cost of dispersal in the harpacticoid copepod, *Tigriopus californicus*. – *Biology Bulletin* 181:123-126.

Chullasorn, S., A. Snidvongs, W. Utoomprukporn & M. Hungspreugs (eds) 1993. Collecting benthic marine invertebrates. – NRCT-JSPS Joint Seminar on Marine Science, Songkhla (Thailand), 2-3 Dec 1993; NRCT-JSPS Joint Seminar on Marine Science, p. 257; Chulalongkorn University, Bangkok, Thailand.

Dethier, M.N. 1980. Tidepools as refuges: Predation and the limits of the harpacticoid copepod *Tigriopus californicus* (Baker). – *Journal of Experimental Marine Biology and Ecology* 42:92-111.

Dybdahl, M.F. 1995. Selection on life-history traits across a wave exposure gradient in the tidepool copepod *Tigriopus californicus* (Baker). – *Journal of Experimental Marine Biology and Ecology* 192:195-210.

Egloff, D.A. 1966. *Biological aspects of sex ratio in experimental and field populations of the marine copepod* Tigriopus californicus. – Ph.D. thesis, Stanford University, Stanford, California.

Fraser, J.H. 1936. The distribution of rock pool copepoda according to tidal level. – *Journal of Animal Ecology* 5:23-28.

Harris, R.P. 1973. Feeding, growth, reproduction and nitrogen utilization by the harpacticoid copepod *Tigriopus brevicornis*. – *Journal of the Marine Biological Association of the United Kingdom* 35:785-800.

Hicks, G.R.F. & B.C. Coull 1983. The ecology of marine meiobenthic harpacticoid copepods. – *Oceanography and Marine Biology Annual Review* 21:67-175.

Hunt, H., R. Scheibling, J.P. Grassle, A. Kelsey, E. Oates & P.V. Snelgrove (eds) 1995. *Settlement patterns of intertidal mussels on artificial collectors and natural substrata.* – Benthic Ecology Meeting, New Brunswick, NJ (USA), 17-19 Mar 1995.

Mahon, R. & S.D. Mahon 1994. Structure and resilience of a tidepool fish assemblage at Barbados. – *Environmental Biology of Fishes* 41:171-190.

Metaxas, A. & R.E. Scheibling, 1993. Community structure and organization of tidepools. – *Marine Ecology Progress Series* 98:187-198.

Metaxas, A. & R.E. Scheibling 1996. Spatial heterogeneity of phytoplankton assemblages in tidepools: Effects of abiotic and biotic factors. – *Marine Ecology Progress Series* 130:179-199.

Monk, C.R. 1941. Marine harpacticoid copepods from California. – *Transactions of the American Microscopical Society* 60:75-99.

Powlik, J.J., M.A. St. John & R.W. Blake 1991. A review of zooplankton pump systems, with notes on the comparative efficiency of towed nets. – *Journal of Plankton Research*, U.K. 13:901-912.

Powlik, J.J. & A.G. Lewis 1996. Desiccation resistance in *Tigriopus californicus* (Copepoda, Harpacticoida). – *Estuarine, Coastal and Shelf Science* 43:521-532.

Powlik, J.J., A.G. Lewis & M. Spaeth 1997. Development, body length, and feeding of *Tigriopus californicus* (Copepoda: Harpacticoida) in laboratory culture and field populations. – *Crustaceana* 70:324-343.

Tablado, A., J.J. Lopez Gappa & N.H. Magaldi 1994. Growth of the pulmonate limpet *Siphonaria lessoni* (Blainville) in a rocky intertidal area affected by sewage pollution. – *Journal of Experimental Marine Biology and Ecology* 175:211-226.

Vittor, B.A. 1971. *Effects of the environment on fitness-related life history characters in* Tigriopus californicus. – Ph.D. thesis, University of Oregon, Eugene.

Zar, J.H. 1984. *Biostatistical Analysis*. Second Edition. – Prentice-Hall, Englewood Cliffs, N.J. 718 pp.

Accepted 22 September 1997

Editorial responsibility: Ulf Båmstedt