**Effect of net size on estimates of abundance, size, age and sex ratio of *Mysis diluviana***

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**Abstract**

We compared catches of *Mysis diluviana* in 80 vertical tows with large (1.0 m diameter) and small (0.5 m diameter) plankton nets to determine if the small net could be used in long-term monitoring historically conducted with the large net. Both nets were constructed of 0.500 mm aperture mesh and were towed simultaneously at 0.4 m/s. Comparisons were made at each of 10 sites on four dates during July-September, 2014 at Dillon Reservoir, Colorado. Estimates of population characteristics (abundance, size structure, and sex ratio) were not different between the two nets. We conclude that the two nets can be used interchangeably; the smaller net is more useful for studies with gear size and weight constraints, but the larger net provides a four times larger sample size and thus may be better for detecting rare individuals.

**Keywords**

Mysis sampling, opossum shrimp, net efficiency, plankton net

**Introduction**

*Mysis*spp. are small (< 25 mm in body length) shrimp-like crustaceans. Two closely related and ecologically analogous species, *M. diluviana* and *M. relicta*, are native to deep, cold freshwater lakes of North America and Europe, respectively (Audzijonyte and Vainola, 2005). Only recently were these two taxa considered separate species (Audzijonyte and Vainola, 2005). Both species are omnivorous and perform diel vertical migrations, inhabiting benthic habitat during the day and migrating into the pelagic to feed on plankton after sunset (Beeton and Bowers, 1982; Grossnickle, 1982). Mysids can be very abundant (> 1,000 individuals/ m2) even in oligotrophic waters (Caldwell and Wilhelm, 2012; Grossnickle and Morgan, 1979) and hence play important roles in trophic dynamics of host systems (Rudstam and Johannsson, 2009). Both species were widely introduced outside their native ranges by fisheries managers during the twentieth century with unexpected and generally negative impacts (Lasenby et al., 1986; Nesler and Bergersen, 1991). Instead of providing a new food source for sport fish, anti-predation behavior allowed introduced *Mysis* to avoid most piscine predators, and they competed with fish for zooplankton (Lasenby et al., 1986; Northcote, 1991). Direct and indirect effects of *Mysis* introductions resulted in the collapse of numerous salmonid fisheries in the western United States (Martinez et al., 2009). Today, regular sampling of mysid populations is necessary to understand and manage their role in food webs, effects on water quality, and competition with fish populations (Caldwell and Wilhelm, 2012; Ellisa et al., 2011; Johnson and Martinez, 2012).

Quantitative sampling of mysids is complicated by their association with the substrate by day where they may be difficult to observe or capture, and their movements in the water column at night. Accordingly, mysid populations have been sampled with different methods at different times of day. During daytime, quadrat counts (Lasenby, 1971) and epibenthic sleds or trawls (Furst, 1972; Maiolie and Bergersen, 1991) have been used but such methods can underestimate abundance compared with net tows at night when mysids are pelagic (Grossnickle and Morgan, 1979; Nero and Davies, 1982). While nocturnal vertical tows with plankton nets appear to be the most common sampling approach, an informal survey of the literature showed that a variety of net configurations that could differ in their sampling efficiency and selectivity have been used (Table 1). Most investigators have used simple conical plankton nets but Bongo, pyramidal, and closing nets have been used. Net opening diameters of 0.30 (Griffiths, 2007; Kjellberg et al., 1991) to 1.08 m (Brownell, 1970) have been used but the most common diameter was 1.00 m, followed by 0.50 m diameter. Net lengths were only reported in a third of studies but ranged 1.4 -5.0 m. Mesh aperture sizes ranged 0.130 mm (Lehman et al., 1990) to 1.350 mm (Rumsey, 1985) with 0.500 mm being the most common mesh. Very few investigators reported tow speed (range: 0.3 -1.0 m/s) or using flow meters to measure filtration volume.

Because net dimensions, mesh aperture size, and tow speed all affect the performance of plankton nets (De Bernardi, 1984), the lack of standardized sampling protocols makes comparisons among *Mysis* studies difficult. Controlled studies that evaluate the effects of net configuration on estimates of population characteristics are needed to identify potential biases due to sampling methodology. In this study we compared the catch from two commonly used *Mysis* nets, testing for differences in population density (n/m2), size and stage structure, and sex ratio. Comparisons were repeated over a three month period to account for possible differences in *Mysis* demographics or ambient conditions that could affect sampling characteristics of the nets.

**Methods**

Sampling was conducted at Dillon Reservoir, a large (1,335 ha) montane (2,750 m ASL) reservoir in central Colorado (39°36.554’ N 106°03.665’ W) (Fig. 1). Mean and maximum depths are 23 m and 66 m, respectively. Dillon Reservoir has been characterized as mesotrophic (summer total phosphorus = 6 μg/L, chlorophyll-a = 7 μg/L, Secchi depth = 3.4 m) (Lewis et al., 1984; Johnson, unpublished data). The reservoir is dimictic and ice-free during May through mid-November. Surface temperatures rarely exceed 18 °C and oxygen concentrations below 4 mg/L have not been observed (Lewis et al., 1984; Johnson, unpublished data). *Mysis diluviana* were introduced into Dillon Reservoir in 1970 and established a large population throughout the reservoir (Martinez et al., 2010).The population exhibits a one-year life cycle, with some individuals attaining a maximum length of about 24 mm by late fall.

We sampled with two net sizes. Each conical net had 0.500 mm aperture Nitex mesh attached to a steel ring. Three lines connected the steel ring to a central attachment point for a single tow rope. Each net terminated with a removable cup with 0.500 mm Nitex mesh. The larger net had a diameter of 1.0 m and was 3.0 m long. This net was adopted for standardized sampling of Colorado’s Mysis populations in 1991 (Martinez, 1992) and was used to sample 14 large reservoirs regularly during 1991-2009 (Martinez et al., 2010). The smaller net had a diameter of 0.5 m and was 2.0 m long. We developed this net for sampling in remote locations where nets needed to be towed by hand from a small raft. Because we were interested in maintaining compatibility with the historic database, we needed to know if the smaller net had similar sampling characteristics as the large net. To test this we determined filtration efficiency of each net and then sampled with both nets simultaneously in a reservoir with Mysis density close to the statewide average and compared resulting population density (n/m2), size structure, life stage composition, and sex ratio.

Mysis sampling took place on two consecutive nights in July (results pooled) and on single nights in August and September, 2014. Sampling stations coincided with those of Martinez et al. (2010).The 10 stations were selected from within three depth strata (<20 m, 20-40 m, and >40 m) and represented all of the major basins and regions of the reservoir (Fig. 1). Sampling commenced at least 60 min after sunset during periods with no moon. Each net was deployed on its own davit, about 3 m apart. The nets were lowered simultaneously until the cups were within 1 m of the bottom, as guided by a depth sounder. Nets were allowed to rest for 60 sec and then retrieved simultaneously at a constant rate of 0.4 m/s with electric winches. We collected one sample with each net type at each of the 10 stations. The catch from each haul was preserved in 70% ethanol.

In the laboratory samples of mysids were transferred to distilled water and examined under a stereomicroscope at 7x magnification. Each individual was counted and classified as 1) juvenile (< 10 mm; Pothoven et al., 2000), 3) male (extended pleopods, Balcer et al., 1984), 4) female (brood pouch exposed), or 5) adult of undetermined gender (>10 mm and neither gravid nor male). Each mysid was measured (nearest 0.1 mm) along a dorsal line from the tip of the rostrum to the tip of the telson using a calibrated micrometer.

Total counts of the catch in each net sample were normalized to number/m2 based on the cross-sectional area of the net openings. Statistical analyses were performed with α = 0.05. Abundance and sex ratio data were not normally distributed so we constructed negative binomial regression models to test for differences in the number of Mysids captured by the two nets controlling for sampling date, age class, sex, and their interactions. To test for differences in the proportion of juveniles captured between the two net sizes and across sampling dates we constructed a beta regression model controlling for sampling date, age class, and their interactions. We tested for effects of net size and sampling date on the mean length of the catch using robust linear regression that reduces the influence of outliers while controlling for sampling date, age class, sex, and their interactions.

**Results**

Mysis density, and proportion of juveniles were highly variable across sites in each sampling month and in both nets, and the distributions of catches were all positively skewed (Fig. 2). Mean density during the study was 232.7 ± 163 mysids/m2 (±SD), similar to the long term average for the reservoir (247.5 ± 106.9 mysids/m2) (B. Johnson, unpublished data). While there was no apparent temporal trend in density, mean length increased with time and the density of juveniles decreased with time, as would be expected for a population sampled during the growing season, post reproduction. The female:male ratio was relatively stable over the course of the study and averaged 0.541 ± 0.335. The relative frequency of adults of indeterminate sex increased during the study (p = 0.008; Fig. 3).

There was no evidence for an effect of net size or sampling date on the number of mysids caught (p = 0.955, and p = 0.157, respectively) nor were there significant interactions between net size and sampling date (p ≥ 0.424). There was no evidence for an effect of net size or sampling date on the number of mysids of each sex caught (p = 0.636, and p = 0.513, respectively) nor were there significant interactions between net size and sampling date (p ≥ 0.142). We found no evidence for an effect of net size on the proportion of juveniles in the catch (p = 0.783). We did find a significant effect of net size on mean length (p = 0.006) after controlling for sampling date, sex, and their interaction. However, the sample size was large (n = 9,127) so our power to detect differences was high, and the effect size was small (0.21 mm) relative to the overall mean length (10.35 mm). A difference of this magnitude is not of practical interest. Qualitatively, the monthly length-frequency distributions were similar between the two nets (Fig. 3). Catches from both nets had equivalent modes, but the range of sizes captured was slightly greater in the large net. The large net consistently caught more mysids ≥ 20 mm TL than the small net (Fig. 3), but these individuals were a tiny fraction of the total sample (mean = 0.75% in large net).

**Discussion**

We found no difference in most characteristics of the *Mysis* population measured with 1.0 m and 0.5 m diameter plankton nets with identical mesh size and towed at equal speed. While it did appear that the large net sampled a slightly broader size distribution than the small net, differences were probably not biologically relevant. Thus, these two nets can be used interchangeably without introducing sampling bias due to net size effects. This conclusion is robust considering the fact that we performed a large number of paired comparisons covering most of the limnetic area of the reservoir over a three month period. Our results are consistent with Kjellberg et al. (1991) who reported that density and size structure of *Mysis relicta* were comparable in 0.3-m and 1.0-m nets. Apparently, sampling efficiency of Mysis nets, the ratio of the number of organisms captured to the number of organisms present in the volume swept by the net (De Bernardi, 1984), does not differ over a relatively broad range of net opening sizes.

Efficiency of plankton nets is also a function of filtration efficiency (the ratio of the volume of water passed through the net to the volume of water that would pass if there was no resistance to water flow). The ratio of the filtering area to the area of the net opening affects filtration efficiency, as does the size and abundance of particles in the water which can clog pores in the mesh. Tranter and Heron (1967) found that in general plankton nets needed a filtering area:opening area >3:1 for ≥ 85% filtration efficiency and > 5:1 for 95% efficiency. Our large net had a ratio of 7:1 and the small net had a ratio of 9:1 so both nets should have high filtration efficiency in the absence of mesh clogging effects. The 0.500 mm aperture mesh we used probably reduces clogging by phytoplankton while preventing loss of small mysids possible with larger mesh apertures (Martinez, 1992).

Our choice of mesh aperture and tow speed are similar to those used in other Mysis studies (mean = 0.569 mm, and 0.43 m/s, respectively; Table 1) so our findings should be relevant to other investigators. While there have been few designed comparisons of Mysis net configurations, existing evaluations also support the mesh and tow speed we used. Chipps and Bennett (1996) reported that densities of juvenile and adult mysids and length-frequency distributions were similar in 0.33 mm and 1.0 mm mesh nets towed at a speed similar to ours (0.44 m/s). Nero and Davies (1982) found that catches were not different at tow speeds of 0.125 - 0.5 m/s. Together, these studies suggest that our results may be applicable across a range of mesh sizes and tow speeds that encompass most of the range of each reported in the literature (Table 1) .

Our conclusions about effects of net size should also be applicable to other Mysis populations. We performed our comparisons over a growing season when the age and size structure of the population changed and yet these temporal changes had no effect on the outcome of the net comparisons. While the larger net captured more large (≥ 20 mm) individuals than the smaller net, it is not known if this was a simple random effect of the 4x larger sample size gathered by the larger net being more likely to detect rare individuals, or if there was net avoidance by large mysids. The fact that the large net also detected rare, smaller individuals that the small net did not supports the former hypothesis. Regardless, few *Mysis diluviana/M. relicta* populations are reported to have many individuals ≥ 20 mm (Ball et al., 2015; Beeton and Gannon, 1991; Caldwell and Wilhelm, 2012; Carpenter et al., 1974; Furst, 1972; Kjellberg et al., 1991; Scharf and Koschel, 2004; Tattersall and Tattersal, 1951). Still, investigators specifically interested in rare individuals may wish to use the larger net to enhance sampling probability.

**Conclusions**

We found no biologically relevant differences in *Mysis* population characteristics measured with 1.0 m and 0.5 m diameter plankton nets with identical mesh size and towed at equal speed. Thus, the choice of net size can be dictated by practical constraints and the research questions of interest. When gear size and weight are important considerations, for example when sampling in remote locations or from small boats, the smaller diameter plankton net can be used without bias compared with a 1.0 m net. When size and weight constraints allow, the larger diameter net may be preferable because it captures approximately four times the size of sample obtained from the half meter net and therefore is more likely to sample rare individuals.

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Table 1. Configurations of vertical tow nets used for sampling *Mysis* *diluviana* or *M. relicta* populations from an informal survey of the literature. NR = not reported. Only one instance of a net type/investigator/location was recorded to reflect the diversity of approaches among investigators.

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| --- | --- | --- | --- | --- | --- | --- |
| Net type | Net mouth size (m) | Net length (m) | Mesh size (mm) | Tow speed (m/s) | Reported by | Location |
| Bongo net | 0.50 | NR | 0.571 | NR | Shea and Makarewicz 1989 | Lake Ontario |
| Bongo net | 0.75 | NR | 0.202 | NR | Richards et al. 1991 | Lake Tahoe, CA-NV |
| Bongo net | 0.75 | NR | 0.500 | NR | Morgan 1980 | Lake Tahoe, CA-NV |
| Closing net | 1.00 | NR | 0.130 | NR | Lehman et al. 1990 | Lake Michigan |
| Closing net | 1.00 | NR | 0.300 | NR | Lehman et al. 1990 | Lake Michigan |
| Closing net | 1.00 | NR | 1.000 | 0.30 | Rudstam et al. 2008 | Lake Ontario |
| Closing net | 1.00 | NR | 0.500 | NR | Næsje et al. 1991 | Lake Jonsvatn, Norway |
| Closing net | 1.00 | NR | 0.500 | NR | Spencer et al. 1999 | Flathead Lake, MT |
| Conical plankton | 0.30 | NR | 0.250 | NR | Griffiths 2007 | Lough Neagh, Northern Ireland |
| Conical plankton | 0.40 | 1.4 | 0.150 | 0.65 | Scharf and Koschel 2004 | Feldberg Lake District, Germany |
| Conical plankton | 0.41 | NR | 0.405 | NR | Bagge et al. 1996 | Lake Saimaa, Finland |
| Conical plankton | 0.84 | NR | 0.405 | NR | Bagge et al. 1996 | Lake Saimaa, Finland |
| Conical plankton | 0.50 | NR | 0.183 | NR | Liljendahl-Nurminen et al. 2008 | Lake Hiidenvesi, Finland |
| Conical plankton | 0.50 | NR | 0.250 | 1.00 | Ahrenstorff et al. 2011 | Lake Superior |
| Conical plankton | 0.50 | NR | 0.250 | 0.50 | Ball et al. 2015 | Lake Champlain, VT |
| Conical plankton | 0.50 | 2.0 | 0.333 | 0.44 | Chipps and Bennett 1996 | Lake Pend Oreille, ID |
| Conical plankton | 0.50 | 2.0 | 1.000 | 0.44 | Chipps and Bennett 1996 | Lake Pend Oreille, ID |
| Conical plankton | 0.65 | NR | 0.800 | NR | Langeland et al. 1991a | Inland lakes in Ontario, Norway |
| Conical plankton | 0.73 | 2.3 | 1.000 | 0.30 | Watkins et al. 2015 | Lake Ontario |
| Conical plankton | 0.75 | 5.0 | 0.285 | NR | Foster and Sprules 2009 | 8 inland lakes, Ontario |
| Conical plankton | 0.75 | NR | 0.500 | NR | Paterson et al. 2011 | Lakes 373 and 375, Ontario |
| Conical plankton | 0.75 | NR | 0.571 | 0.33 | Grossnickle and Morgan 1979 | Lake Michigan |
| Conical plankton | 0.75 | NR | 0.571 | NR | Madeira et al. 1982 | Lake Michigan |
| Conical plankton | 1.00 | 3.0 | 0.500 | 0.37 | Martinez et al. 2010 | Colorado reservoirs |
| Conical plankton | 1.08 | 2.5 | 0.570 | NR | Brownell 1970 | Cayuga Lake, NY |
| Conical plankton | 1.00 | 3.0 | 1.000 | 0.33 | Caldwell and Wilhelm 2012 | Lake Pend Oreille, ID |
| Conical plankton | 1.00 | NR | 1.000 | 0.30 | Gal et al. 1999 | Cayuga Lake, NY, Lake Ontario |
| Conical plankton | 1.00 | 3.0 | 1.000 | 0.50 | Pothoven et al. 2010 | Lake Michigan |
| Conical plankton | 1.00 | 3.0 | 1.350 | 0.35 | Rumsey 1985 | Western MT lakes |
| Conical plankton | 1.50 | NR | 1.050 | 0.50 | Bowers and Vanderploeg 1982 | Lake Michigan |
| “framed net” | 0.50 | NR | 0.243 | NR | McDonald et al. 1990 | Lake Michigan |
| Inverted pyramid | 1.00 | NR | 0.505 | NR | Carpenter et al. 1974 | Laurentian Great Lakes |
| Inverted pyramid | 1.00 | 1.5 | 1.000 | 0.44 | Johannsson 1992 | Lake Ontario |
| Inverted pyramid | 1.00 | NR | 0.500 | NR | Koksvik et al. 2009 | Lake Jonsvatn, Norway |
| Inverted pyramid | 1.00 | 1.5 | 1.000 | 0.33 | Nero and Davies 1982 | Lake 223, Ontario, Canada |
| Inverted pyramid | 1.00 | NR | 1.000 | 0.30 | Stockwell et al. 2014 | Lake Superior |
| Wisconsin net | 1.00 | NR | 0.500 | NR | Beattie and Clancy 1991 | Flathead Lake, MT |
| NR | 0.30 | NR | 0.200 | NR | Kjellberg et al. 1991 | Lake Mjøsa, Norway |
| NR | 1.00 | NR | 0.200 | NR | Kjellberg et al. 1991 | Lake Mjøsa, Norway |
| NR | 1.00 | NR | 0.500 | NR | Langeland et al. 1991b | Lake Selbusjøen, Norway |
| Mean | 0.81 | 2.5 | 0.569 | 0.43 |  |  |

**Figure Captions**

Figure 1. Ten locations on Dillon Reservoir, Colorado sampled with simultaneous tows of 0.5 m and 1.0 m diameter plankton nets.

Figure 2. Density (a), proportion of juveniles (b), mean length (c), and sex ratio (d) of mysids sampled with 0.5 m diameter (gray) and 1.0 m diameter (white) plankton nets at Dillon Reservoir, Colorado.

Figure 3. Length-frequency distributions and life stage/sex composition (pie charts) of Mysis diluviana sampled with 0.5 m diameter (upper panels) and 1.0 m diameter (lower panels) plankton nets during three months in 2014 at Dillon Reservoir, Colorado. Sample size (N) is also shown.