**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 500-µm aperture mesh and were towed simultaneously at 0.4 m/s. Comparisons were made at each of 10 sites on three occasions during July-September, 2014 at Dillon Reservoir, Colorado. Estimates of abundance and sex ratio were not different between the two nets but the larger net sampled a slightly broader range of sizes than the smaller net. We conclude that for most population monitoring purposes, 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, 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). Both species are omnivorous and perform diel vertical migrations, inhabiting deeper water during the day and migrating up to feed on plankton after sunset (Beeton and Bowers, 1982). Only recently were these two taxa considered separate species (Audzijonyte and Vainola, 2005); we assumed their sampling characteristics are similar. Mysids can be very abundant (> 1,000 individuals/ m2) even in oligotrophic waters (Caldwell and Wilhelm, 2012) 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 (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 (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; 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 (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, closing, pyramidal, and Wisconsin nets have been used. Net opening diameters of 0.30 m (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 (Table 1). Net lengths were only reported in a third of studies but ranged from 1.4 -5.0 m. Mesh aperture sizes ranged 130-µm (Lehman et al., 1990) to 1350-µm (Rumsey, 1985) with 500-µm and 1000-µm being the most common mesh sizes (Table 1). 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 (individuals/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 (1335 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 500-µm 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 500-µm 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 sampled with both nets simultaneously in a reservoir with *Mysis* density close to the statewide average and compared resulting population density (individuals/m2), size structure, life stage composition, and sex ratio.

*Mysis* sampling took place on two consecutive nights in July (n = 20 paired tows) results pooled) and on single nights in August (n = 10 paired tows) and September (n = 10 paired tows), 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 s 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), 2) male (extended pleopods, Balcer et al., 1984), 3) female (brood pouch exposed), or 4) adult of undetermined sex (>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 individuals/m2 based on the cross-sectional area of the net openings. Statistical analyses were performed using the R program for statistical computing (R Core Team 2014), with α = 0.05. For further details and R code of the analysis, see online appendix at <http://jtipton25.github.io/mysis/>. We had three main questions of interest: 1) Is there a difference in density of mysids caught between the two nets?, 2) Is there a difference in the length of mysids caught between the two nets?, and 3) Does the density, broken down by age and sex class, differ between the two nets?

Because density estimates cannot be less than zero and often are right skewed, the assumption of normality in the data was questionable. Hence, methods like linear regression, t-tests, and ANOVA that assume normal distributions were not ideal, but, for completeness we began with a paired t-test. We tested for a difference in density between the nets more formally by fitting a generalized linear model (GLM) controlling for covariates (date of sampling and sampling location). The GLM approach did not require the assumption of normality. Instead, we constructed a negative binomial model

where is the mean of the negative binomial distribution for sampling event and is an overdispersion parameter that allows for the mean and variance to be different. We modeled with covariates using the canonical log link function

where is the set of covariates for observation . Then we performed inference on our coefficients , where the interpretation of is a percent change in density per unit change in . Regression coefficients for each model are presented in the Appendix.

One could argue that because the larger net catches four times the number of *Mysids*, the large net is more likely to catch mysids at the extremes of the size classes (either really small or really large mysids). To account for this, we performed a paired t-test using a trimmed mean, removing the smallest and largest 5% of the lengths before calculating the sample mean. To better account for the different sample sizes (the group means are from unbalanced sample sizes), we fit a linear model and a robust linear model that assumes overdispersion in the data. We started by checking for heterogeneity in the group variances using Bartlett’s and Levene’s tests. To test if there was a difference in length between net sizes while accounting for heterogeneity, we used a weighted least squares regression where the weights are the inverse of the group variances, as well as a robust linear regression. As a final analysis, we applied a robust regression, using an M-estimator model that accounts for heterogeneity in variance and presence of outlying observations.

To test for a change in counts broken down by sex class between the two net sizes, we constructed a negative binomial regression model that examined the effects of net size, date of sampling, sampling location, and sex class. We also tested if the counts within a sex class were different between the net sizes by constructing a negative binomial model that controlled for sampling date and station. We also examine if the counts within a sex class are different between the net sizes. To test this we constructed a negative binomial model that controls for sampling date and station.

**Results**

*Mysis* density was highly variable across sites in each sampling month and in both nets. The distributions of catches appeared non-normal and were positively skewed, and there was no apparent temporal trend in density (Fig. 2). Mean density during the study was 233 ± 163 individuals/m2 (±SD), similar to the long term average for the reservoir (248 ± 107 individuals/m2) (Martinez et al. 2010).

The paired t-test found no significant difference (*t*-value = 0.069, *df* = 39, *p*-value = 0.945) in density between the two nets. The results shown below show that there is no evidence of an effect on the number of counts observed between the two net size s (*z*-value = 0.095, *p*-value=0.924).

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. However, the shapes of the length distributions by net size had equivalent modes, and overall they look quite similar during each sampling period (Fig. 3) suggesting that there is not a difference in length distribution between nets across time. The range of sizes captured was slightly greater in the large net (Fig. 3) but these small and large individuals constituted < 1% of the total catches with the large net. The distributions of lengths between the net sizes with respect to sex class look quite similar as well (Fig. 4), suggesting that sex did not affect the comparison of lengths between the two nets. The histogram of paired differences shows slight evidence that the smaller net caught smaller mysids, although the distribution appears to be centered near 0. The paired t-test found a significant difference (*t*-value = -2.158, *df* = 39, *p*-value = 0.037) in expected counts but effect size was quite small (-0.293 mm), and the paired t-test using the trimmed means did not find a significant difference (*t*-value = -1.754, *df* = 39, *p*-value = 0.087) in expected counts.

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The distribution of sampling variance by sampling occasion shows some difference in variance of lengths by sampling occasion and Bartlett's test (*p*-value < 0.001) and Levene's test (*p*-values < 0.001).

Weighted least squares regression also found a statistically significant difference in mean mysid length between the two nets (*t*-value = 2.134, *p*-value = 0.033) but sample size was large (n = 9127) so our power to detect differences was high. After accounting for the location, date, sex distribution, and heterogeneity of variance the difference in mean length between the two net sizes was very small (0.086 mm). The robust linear model also found statistically significant differences in mean length between the net sizes (*t*-value = 2.09, *p*-value = 0.037, *df* = 9111), but again the effect size was small (0.078 mm). Another measure of effect size is Cohen's *d* which measures the difference in means relative to a pooled standard deviation. For our data, Cohen's *d* = 0.075, which implies that the effect of net size is quite small.

The female:male ratio was relatively stable over the course of the study (mean = 0.541 ± 0.335) and was similar between the two net sizes (Fig. 2). The relative frequency of adults of indeterminate sex increased during the study (*p*-value = 0.008; Fig. 3) as juveniles achieved adult size (> 10 mm) but had not yet begun expressing sexual characteristics. A visual inspection of density by sex class showed no difference in counts by net size, but does show a change in counts over time for each sex class (Figure 5).

The negative binomial regression model showed that there was no effect of net size on density of sex classes, when controlling for sampling date, sampling location, and sex class (*z*-value = 0.185, *p*-value = 0.853). The negative binomial model that controlled for sampling date and station showed that time of year and location were important in influencing juvenile density estimates, but net size was not (*z*-value = -0.731, *p*-value = 0.465). Similar negative binomial regression models for counts of males and females showed similar effects (*z*-value = -0.571, *p*-value = 0.568 and *z*-value = 1.607, *p*-value = 0.108 for males and females, respectively).

**Discussion**

We found few differences in 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. This finding is robust considering the fact that we performed a large number of paired comparisons at locations covering most of the limnetic area of the reservoir. The larger net sampled a slightly broader size distribution of mysids than the smaller net. This result could be due to differences in filtration efficiencies, net avoidance, sampling area of the two nets, or some combination of these factors.

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) of our nets were probably not very different. The ratio of the filtering area to the area of the net opening affects filtration efficiency, as do mesh fiber diameter and the size and abundance of particles in the water which can clog pores in the mesh. Only the first factor differed between nets in our study. 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.

While the large net captured more large (≥ 20 mm) individuals than the small net, it is not known if there was net avoidance by large mysids or this result was simply due to the 4x larger sample size gathered by the larger net being more likely to detect rare individuals. Both factors may have been operating since large mysids are more powerful swimmers and thus, could more easily avoid a small net, but the large net also detected more very small and rare individuals so sampling area was also important. Regardless, these small and large individuals constituted a tiny fraction of the catches, and few *Mysis diluviana/M. relicta* populations are reported to have many individuals ≥ 20 mm (Ball et al., 2015; Caldwell and Wilhelm, 2012; Carpenter et al., 1974; Kjellberg et al., 1991). Still, investigators specifically interested in rare individuals may wish to use the larger net to enhance sampling probability. For example, studies aimed at capturing adult mysids for estimating egg production rates may wish to use larger nets.

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 greatly over a relatively broad range of net opening sizes. Our choice of mesh aperture and tow speed are similar to those used in other *Mysis* studies (mean = 569-µm, 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 330-µm and 1000-µm 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 findings about effects of net size may 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. It should be noted that *Mysis* abundance in Dillon Reservoir was relatively high, and we did not sample during isothermal conditions. Net size effects may be different for lower density populations and at other times of the year.

We conclude that for basic population monitoring, the two nets we used can be used interchangeably without introducing sampling bias due to net size effects. 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 with limited bias compared with the 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 small net and therefore the large net is more likely to sample rare individuals that may be of scientific interest.

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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. “Bongo” is a paired conical plankton net, “closing” is a plankton net with closing mechanism, “conical” is a simple plankton net, “pyramid” is an inverted pyramid shaped net, and “Wisconsin” is a high efficiency plankton net.

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| --- | --- | --- | --- | --- | --- | --- |
| Net type | Net mouth size (m) | Net length (m) | Mesh size (µm) | Tow speed (m/s) | Reported by | Location |
| Bongo | 0.50 | NR | 571 | NR | Shea and Makarewicz 1989 | Lake Ontario |
| Bongo | 0.75 | NR | 202 | NR | Richards et al. 1991 | Lake Tahoe, CA-NV |
| Bongo | 0.75 | NR | 500 | NR | Morgan 1980 | Lake Tahoe, CA-NV |
| Closing | 1.00 | NR | 130 | NR | Lehman et al. 1990 | Lake Michigan |
| Closing | 1.00 | NR | 300 | NR | Lehman et al. 1990 | Lake Michigan |
| Closing | 1.00 | NR | 1000 | 0.30 | Rudstam et al. 2008 | Lake Ontario |
| Closing | 1.00 | NR | 500 | NR | Næsje et al. 1991 | Lake Jonsvatn, Norway |
| Closing | 1.00 | NR | 500 | NR | Spencer et al. 1999 | Flathead Lake, MT |
| Conical | 0.30 | NR | 250 | NR | Griffiths 2007 | Lough Neagh, Northern Ireland |
| Conical | 0.40 | 1.4 | 150 | 0.65 | Scharf and Koschel 2004 | Feldberg Lake District, Germany |
| Conical | 0.41 | NR | 405 | NR | Bagge et al. 1996 | Lake Saimaa, Finland |
| Conical | 0.84 | NR | 405 | NR | Bagge et al. 1996 | Lake Saimaa, Finland |
| Conical | 0.50 | NR | 183 | NR | Liljendahl-Nurminen et al. 2008 | Lake Hiidenvesi, Finland |
| Conical | 0.50 | NR | 250 | 1.00 | Ahrenstorff et al. 2011 | Lake Superior |
| Conical | 0.50 | NR | 250 | 0.50 | Ball et al. 2015 | Lake Champlain, VT |
| Conical | 0.50 | 2.0 | 333 | 0.44 | Chipps and Bennett 1996 | Lake Pend Oreille, ID |
| Conical | 0.50 | 2.0 | 1000 | 0.44 | Chipps and Bennett 1996 | Lake Pend Oreille, ID |
| Conical | 0.65 | NR | 800 | NR | Langeland et al. 1991a | Inland lakes in Ontario, Norway |
| Conical | 0.73 | 2.3 | 1000 | 0.30 | Watkins et al. 2015 | Lake Ontario |
| Conical | 0.75 | 5.0 | 285 | NR | Foster and Sprules 2009 | 8 inland lakes, Ontario |
| Conical | 0.75 | NR | 500 | NR | Paterson et al. 2011 | Lakes 373 and 375, Ontario |
| Conical | 0.75 | NR | 571 | 0.33 | Grossnickle and Morgan 1979 | Lake Michigan |
| Conical | 0.75 | NR | 571 | NR | Madeira et al. 1982 | Lake Michigan |
| Conical | 1.00 | 3.0 | 500 | 0.37 | Martinez et al. 2010 | Colorado reservoirs |
| Conical | 1.08 | 2.5 | 570 | NR | Brownell 1970 | Cayuga Lake, NY |
| Conical | 1.00 | 3.0 | 1000 | 0.33 | Caldwell and Wilhelm 2012 | Lake Pend Oreille, ID |
| Conical | 1.00 | NR | 1000 | 0.30 | Gal et al. 1999 | Cayuga Lake, NY, Lake Ontario |
| Conical | 1.00 | 3.0 | 1000 | 0.50 | Pothoven et al. 2010 | Lake Michigan |
| Conical | 1.00 | 3.0 | 1350 | 0.35 | Rumsey 1985 | Western MT lakes |
| Conical | 1.50 | NR | 1050 | 0.50 | Bowers and Vanderploeg 1982 | Lake Michigan |
| Pyramid | 1.00 | NR | 505 | NR | Carpenter et al. 1974 | Laurentian Great Lakes |
| Pyramid | 1.00 | 1.5 | 1000 | 0.44 | Johannsson 1992 | Lake Ontario |
| Pyramid | 1.00 | NR | 500 | NR | Koksvik et al. 2009 | Lake Jonsvatn, Norway |
| Pyramid | 1.00 | 1.5 | 1000 | 0.33 | Nero and Davies 1982 | Lake 223, Ontario, Canada |
| Pyramid | 1.00 | NR | 1000 | 0.30 | Stockwell et al. 2014 | Lake Superior |
| Wisconsin | 1.00 | NR | 500 | NR | Beattie and Clancy 1991 | Flathead Lake, MT |
| NR | 0.30 | NR | 200 | NR | Kjellberg et al. 1991 | Lake Mjøsa, Norway |
| NR | 1.00 | NR | 200 | NR | Kjellberg et al. 1991 | Lake Mjøsa, Norway |
| NR | 1.00 | NR | 500 | NR | Langeland et al. 1991b | Lake Selbusjøen, Norway |
| Mean | 0.81 | 2.52 | 578 | 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. Box and whisker diagrams of 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. Horizontal lines within the boxes show the median and the diamonds show the mean.

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.