The use of *Artemia* biomass sampling to predict cyst yields in culture ponds

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**Abstract**

The possibility of using biomass volume (= mean biomass present in the pond.week⁻¹) to predict the total amount of harvestable cysts (= kg wet weight collected. week⁻¹) produced in a culture pond by an *Artemia franciscana* population using a mixed model regression was evaluated for two different sampling methods; horizontal transects and vertical point samples. For transects, the following equation was found: ‘log (0.01 + cyst yields) = –2.05 + 0.025*(biomass volume)’ with \( F(1,4.87) = 8.83 \) and \( p = 0.032 \). For the point samples, the regression was also significant with \( F(1,55.2) = 13.62 \) and \( p = 0.0005 \) for following equation: ‘log (0.01 + cyst yield) = –3.613 + 0.021*(biomass volume). As pond effect and interaction terms did not significantly explain a significant portion of the variance for either of the sampling methods (Transects: pond: \( F(3,14.3) = 2.48; p = 0.103 \); pond*biomass volume: \( F(3,3.61) = 4.63; p = 0.0976 \); Point samples: pond: \( F(3,44.5) = 0.00; p = 0.999 \); pond*biomass volume: \( F(3,44.2) = 0.11; p = 0.954 \)), the variable pond (repeated measurement factor) was not included in the final calculations for the regression equations. Although a combination of factors influences the equation, the high significance levels of the regression indicate biomass volume can be safely used to predict production trends. The low investment requirements of this method make it especially attractive for on farm use, where correctly determining the point of cyst decline will help farmers to allocate resources where needed.

**Introduction**

Previous work with pond cultures has demonstrated that cyst yields steadily decline with time. This is true in the larger salt fields where *Artemia* sp. is cultured on a year-round basis (Camara & De Meideros Rocha, 1987). But even in Vietnam where the culture season is relatively short (December–May) yields decrease (Baert et al., 1997). Given the high initial stocking density and high recruitment rates of the population in these latter semi-intensive systems, one factor negatively influencing cyst yields is increased intraspecific competition. Indeed, Baert et al (1997) showed that later generations in such systems have lower survival rates and produce smaller broods compared to the parental populations. Based on these observations, the multi-cycle system was developed. In the multi-cycle system, ponds are re-stocked several times a year. This allows farmers to use high initial stocking densities while at the same time keeping feeding levels sufficiently high. Once the second generation starts to compete significantly with the parental population, ponds are drained.

One problem encountered when using the multi-cycle culture system though is to correctly determine when ponds should be drained. Ideally, ponds are drained once the parental generation stops producing significant amounts of cyst. But present yields should also be balanced against potential yields produced by newly inoculated populations. As several other factors
besides stocking density and primary production influence yields, this point typically is difficult to predict. The study of Baert et al. (1997) also indicates that per season at least three culture cycles are needed to guarantee maximum yields in the multi-cycle system. In Vietnam where *Artemia* can only be cultured during a limited time of the year, the length of one cycle varies between 4 and 7 weeks. Being able to predict yields for the coming week will help farmers to decide which ponds to keep and which ponds to re-stock.

To accurately predict cyst yields on farm, a statistic has to be identified which firstly can be measured using a simple sampling method applicable under field conditions, secondly allows immediate appraisal of the current state of the *Artemia* sp. population in the culture pond and thirdly requires minimal investment.

Most methods used to sample benthic vertebrates (Elliot, 1977; Southwood, 1995) or plankton (Southwood, 1995) also can be used to sample *Artemia*. Sampling methods for *Artemia* as found in literature fall in one of three categories. Either samples are taken using a sampling box with a given volume such as i.e. Schindler traps (Lenz, 1980; Wear & Haslett, 1985, 1987), a core-sampler having a given diameter (Marchant & Williams, 1977) or a plankton net towed either vertically or horizontally through the water column (Lenz, 1980, Baert et al., 1997).

Schindler traps (Schindler, 1969) and similar sampling devices have the advantage that the volume sampled is known exactly. They also allow sampling at different depths, which makes them particularly useful to study vertical distribution patterns. However, the relatively small volume of such samplers can result in a very high variability of the samples, especially if the population distribution is contagious, which for *Artemia* is often the case (Lenz, 1980). Therefore, to obtain a reliable estimate having a sufficiently small standard error a very large number of samples using a stratified sampling procedure will be needed in most cases.

Tubes or core samplers (Marchant & Williams, 1977) can only be used efficiently in shallow lakes or ponds. In deeper lakes, such samplers only sample the top-layers. Estimated densities based on such samples could deviate considerably from the true population average as *Artemia* can show significant vertical stratification (Lenz, 1980). Furthermore, when using tubes with a large diameter, emptying the tube becomes a rather time consuming activity, while tubes having a small diameter suffer from the same problems as sample boxes.

Plankton nets (Lenz, 1980) have the advantage that large volumes of water can be sampled. Furthermore, when towed vertically through the water, variability due to vertical stratification is eliminated. Similarly, horizontal tows can reduce variability if they cross several patches of a contagiously distributed population (Elliot, 1977). Care should be taken though to select nets having an appropriate mesh size, length and diameter to avoid clogging. Once nets are clogged, animals will be pushed aside by water currents resulting in underestimation of the population. In this study, samples were taken using plankton nets.

Once samples have been collected, different population characteristics can be obtained. Most often samples are counted and counts are used to estimate the number of animals in the pond, although other characteristics such as length, reproductive output etc. also can be determined. Abundance estimates can be used to reconstruct the life-cycle of the animals in the pond, gather information on the distribution and abundance of the population or to study migration and re-colonization (Lenz, 1980; Ramamoorthi & Thangaraj, 1980; Scelzo & Voglar, 1980; Ramanathan & Natarajan, 1987). Theoretically the number of females together with an estimate of the reproductive output also could be used to estimate potential yields. The fact that this method is time consuming and requires a well-equipped laboratory as well as a thorough mathematical knowledge probably explains why this method seldom has been used for this purpose.

Wear & Haslett (1985, 1987) describe an alternative method, which relates sample dry weight to harvestable biomass. As these authors worked with a predominantly ovoviviparous population, they did not relate dry weight to cyst yields. Also, immediate appraisal of the current state of the *Artemia* population is not possible using this method. Furthermore, given the high saline and turbid environment in which *Artemia* lives, a lot of time has to be devoted to cleaning the samples and the determination of dry weight also requires a lot of time as well as a well-equipped laboratory.

A variable that can be determined quickly on site using cheap plastic measuring cones is biomass volume. This technique described by Lenz (1985) has also the advantage over weight measurements in that it is not as much affected by dirt and salt sticking to the animals. In this study, an adaptation of this technique is proposed.
Materials and methods

Data collections

The data were collected at the field station in Vinh Chau (south-east coast of the Mekong Delta, Vietnam), during the 1995 and 1996 dry seasons. Data were collected in ponds in which animals were cultured using the one- or multi-cycle culture system as described by Baert et al. (1997). For this study, we selected four ponds ranging in size from 0.17 to 0.19 hectares. In 1995, too high stocking of two ponds at the start of the culture season resulted in a zero cyst production. As both ponds were also drained before the end of the experiment, the data-sets of these two cycles were considered as not-representative and discarded from the analysis.

All ponds were rectangular and had a peripheral ditch (depth: 45–50 cm; width: 2–4 m). Samples were collected between 6am and 8am. We only sampled the peripheral ditch for following reasons. During part of the culture season, the platform is not, or only a few centimetres, submerged, in which case no representative samples can be collected. Preliminary sampling during periods when the platform was submerged (more than 10 cm) also showed density estimates based on samples taken in the ditch only or samples taken both in the ditch and on the platform did not differ significantly (randomised block ANOVA, \(N = 5, p = 0.47\)).

In 1995, weekly six horizontal transects per pond were collected. Transects were taken using a conical cotton net (mesh size <100 \(\mu\)m; surface area 0.22 m\(^2\), length 60 cm), dragged horizontally over a distance of 7.5 m, while keeping the net continuously submerged (about 10 cm under the water surface). Samples were taken starting in a clockwise direction in each of the four corners and half way the longest side of the pond.

In 1996, each week ten point samples per pond were collected using a square plankton net (mesh size 100 \(\mu\)m; surface area 0.25 m\(^2\)), hauled vertically through the water column. Samples were distributed in the ditch using fixed random sampling.

Once collected, samples were rinsed for about 1 min using tap water. Large debris were removed manually using pincers. Next, the biomass of each sample was separately transferred to a measuring cylinder of 100 mL, 250 mL or 500 mL depending on the amount of biomass present in the sample. If necessary, water was added to the cylinders to assure all biomass was submerged. The samples were allowed to settle for 5 min, before the volume was estimated (1 mL accurately). Finally average volume biomass per sample was converted to biomass present in the pond using the equation: ‘volume biomass in the pond = [volume biomass(volume water sampled)] volume pond’.

The amount of cysts is given as total harvestable amount week\(^{-1}\) expressed in kg wet weight (WW). Cysts were collected daily (morning, afternoon, evening) using a nylon dip net (mesh size <100 \(\mu\)m). Once collected, they are rinsed through a number of nets having different mesh sizes, to remove dirt (Sorgeloos et al., 1986). Finally water is squeezed out and cyst are weighed using an electronic balance (0.1 g accurate). The ratio ‘wet weight:dry weight’ for cysts processed using this method is close to 3 (Nguyen Van Hoa, unpublished data).

Statistical analysis

Before analyses, normality of the residuals was tested using the graphic test given in Statistica 5.5. As the residuals were not normally distributed the total amount of harvestable cysts produced by an *Artemia franciscana* population, was transformed using the equation ‘log\(^*\)(cyst yield + 0.01)’.

The relation between the total harvestable amount of cysts (log-transformed, fixed effect) and biomass volume was analysed using a mixed model regression with the proc MIXED module of SAS 6.12 (Littell et al., 1996). Pond was added as a repeated measure factor (weekly intervals, autoregression order 1). Variances were estimated using the REML (restricted maximum likelihood method) while correct degrees of freedom were determined using the Satherthwaite formula (Littell et al., 1996).

The correlation between female abundance and biomass volume was calculated using the parametric product-moment correlation coefficient (Sokal & Rohlf, 1995).

### Table 1. Significance levels for the different variables using a mixed model regression analysing the 1995 transect data

<table>
<thead>
<tr>
<th>Factor</th>
<th>d.f.</th>
<th>F-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume</td>
<td>1; 48.7</td>
<td>8.83</td>
<td>0.032</td>
</tr>
<tr>
<td>Pond</td>
<td>3; 14.3</td>
<td>2.48</td>
<td>0.103</td>
</tr>
<tr>
<td>Pond*Volume</td>
<td>3; 3.61</td>
<td>4.63</td>
<td>0.0976</td>
</tr>
</tbody>
</table>
Table 2. Significance levels for the different variables using a mixed model regression analysing the 1996 point sample data

<table>
<thead>
<tr>
<th>Factor</th>
<th>d.f.</th>
<th>F-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pond</td>
<td>1.55</td>
<td>13.62</td>
<td>0.0005</td>
</tr>
<tr>
<td>Pond*Volume</td>
<td>3.44</td>
<td>0.00</td>
<td>0.999</td>
</tr>
<tr>
<td>Pond*Volume</td>
<td>3.44</td>
<td>0.11</td>
<td>0.954</td>
</tr>
</tbody>
</table>

Results

Data for the transect samples collected in 1995 yielded the following equation:

\[ \log (0.01 + \text{cyst yields}) = -2.05 + 0.025 \times (\text{biomass volume}) \]

For the point samples collected in 1996 the regression was:

\[ \log (0.01 + \text{cyst yield}) = -3.613 + 0.021 \times (\text{biomass volume}) \]

F-values and p-values for the transect and point samples are summarized in Tables 1 and 2 respectively. As can be seen from these tables, the regression equation for both transect and point samples is significant (\( F_{(1,48.7)} = 8.83 \) and \( p = 0.032 \) and \( F_{(1,55.2)} = 13.62 \) and \( p = 0.0005 \), respectively) be it a better fit of the curve was found using point samples. One possible explanation could be the higher number of samples taken per pond for the point samples. But it was also noted when taking transects that clogging of the net, especially in ponds having a dense *Artemia* population, resulted in a loss of animals as animals were pushed aside. Indeed, an estimate based on transects often underestimated true population abundance by a factor three to five (Baert Peter, unpublished data).

Also interesting to note is the fact that whereas for point samples the repeated measurement factor ‘pond’ as well as the interaction term did not have any influence on the estimates (pond: \( F_{(3,44.5)} = 0.00 \); \( p = 0.999 \); pond*biomass volume: \( F_{(3,44.2)} = 0.11 \); \( p = 0.954 \)), the interaction term for the transect samples was significant at \( p < 0.1 \) (pond: \( F_{(3,14.3)} = 2.48 \); \( p = 0.103 \); pond*biomass volume: \( F_{(3,36.1)} = 4.63 \); \( p = 0.0976 \)). The latter suggests pond attributes might influence abundance estimates if transect samples are taken. One factor differing between ponds is the animal abundance.

The parametric product-moment correlation coefficient (Sokal & Rohlf, 1995) between the number of females present in the pond and the biomass volume was also calculated. For both years, this correlations is highly significant (transect data 1995, \( p<0.001 \); point samples 1996, \( p<0.01 \)). As the number of females is one important factor determining total yields, this relation partly explains why biomass can correctly predict cyst yields.

Discussion

The significance levels of the regression found for the data of both 1995 and 1996 demonstrate that biomass volume can give a good idea of the expected amount of cysts, which will be produced the coming week. An increase in biomass will be accompanied by an increase in cyst yield, while a declining biomass indicates yields will go down. The strong correlation between biomass volume and estimated number of females present in the pond partly explains this.

Nevertheless, the differences observed between the two years shows the sampling methodology, also strongly influences the regression equation. For the transect samples, the influence of pond characteristics cannot be excluded. One factor differing widely between ponds is population abundance. As nets tend to clog easily when taking transect samples over a longer distance and clogging becomes more pronounced in dense populations, population abundance could be an important pond variable explaining inter-pond variation.

Factors, such as brood size and number of broods.week\(^{-1}\), which only have a minor influence on biomass volume, could also significantly influence cyst yields and render the equations unusable at certain times.

Finally, factors influencing sample volume but not cyst yield also influence the equation. The influence of an asymmetric sex ratio can also be quite prominent in bad ponds, as females tend to die more quickly compared to males when stressed (unpublished data). Furthermore, the number of not reproducing females also tends to increase drastically if animals are stressed or cultures are old (unpublished data). This explains, why cyst yields often decrease more quickly at the end of the culture cycle – when food in the pond becomes limiting – than one would expect when using the equations.

Nevertheless, the low investment requirements of this method together with the ease of use, make the described method still a valuable tool for on farm use,
where correctly and quickly determining the point of cyst decline will help farmers to allocate resources where needed. Especially if enough samples are taken at set times (i.e. weekly) using a net with a large mesh size, predictions will allow a quick assessment of the population, even in large salt lakes. Once enough data are collected, a regression line associated with a certain sampling methodology and site can be calculated, which can be used to predict future cyst yields more exactly.

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References


