

Estuarine Research Federation Conference 2007 Poster Presentation

A Simple Staining Method for Differentiating Live and Dead Copepods in Natural Samples

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ZOOPLANKTON CARCASSES:

Zooplankton sampling is an integral part of many estuarine monitoring programs. Accurate census of the zooplankton community is critical for proper understanding of secondary production and trophic processes in the pelagic ecosystems, and the responses of plankton to environmental changes. However, researchers routinely collect, preserve and count their samples without knowledge of the vital state of the zooplankton; as a result, they tend to overlook zooplankton mortality due to starvation, diseases, pollution, harmful algal blooms and other environmental stresses. A few studies in the literature show that non-consumptive mortality (mortality not due to predation) can be significant at times, demonstrating the clear need for quantifying zooplankton carcasses in natural samples (Fig. 1).

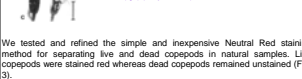
Fig. 1. Lineages reports of occurrence of zooplankton carcasses (as percentage of the total zooplankton).



THE NEUTRAL RED METHOD:

Some researchers identify zooplankton carcasses based on visible loss of internal tissues (Fig. 2), which can be time consuming and difficult for large scale field study.

Fig. 2. This photo shows the difference between an intact and presumably live copepod (right) and a dead copepod with missing internal tissue and visible external mouth (left) (Heary et al. 2002)



We tested and refined the simple and inexpensive Neutral Red staining method for separating live and dead copepods in natural samples. Live copepods were stained red whereas dead copepods remained unstained (Fig. 3).

Fig. 3. Appearance of Neutral Red-treated copepods (dead copepod under a microscope with dark field. Live copepod (2) is stained red, whereas dead copepod (1) remains unstained (Tang et al. 2006)



In laboratory testing, the staining results were not affected by the cause of death, time of death, or staining time (Tables 1 & 2). When properly preserved, stained individuals retained their color for up to 3 months.

Table 1. Percent copepods stained by Neutral Red after different staining methods and staining durations. Unstained natural samples were used as control. Numbers of copepods counted are given in parentheses.	Table 2. Percent copepods stained by Neutral Red after different death and staining durations. Numbers of copepods counted are given in parentheses.																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																
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THE CHESAPEAKE BAY EXPERIENCE:

In field testing, slow (~1 knot) and gentle horizontal tows did not result in significant cod-end mortality (Fig. 3).

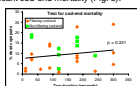


Fig. 3. Percent dead copepods recovered did not change significantly with tow duration, indicating that cod-end mortality occurred via negligible shear (regression: $p < 0.001$). There was also no difference in percent dead copepods between a 15-min tow and a 1-h tow (t-test: cod-end and dead: $p > 0.1$).

Using the Neutral Red method in the summer of 2005, we found that about an average ~29% of the copepods in the lower Chesapeake Bay were dead. Percent dead copepods did not differ between horizontal tows and vertical tows, suggesting that dead copepods were homogeneously distributed in the water column.

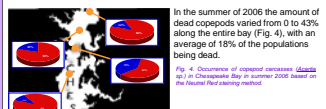


Fig. 4. Occurrence of copepod carcasses (dead) in the Chesapeake Bay in summer 2005 based on the Neutral Red staining method.

Among the environmental parameters measured, only % water column with low dissolved oxygen weakly correlated with the percent dead copepods (Fig. 5).

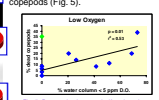


Fig. 5. Percent dead copepods (dead) did not weakly correlated with % water column with low oxygen (< 2 parts per million) (green). No correlation was found for chlorophyll *a* (blue) (staining method).

We also found that turbulence generated by boat motors increased % dead copepods significantly (Fig. 6).

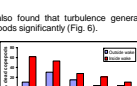
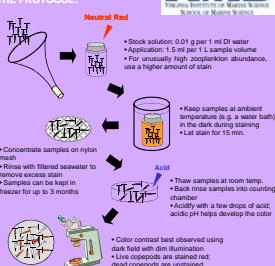


Fig. 6. Live collected freshwater net tow results (left) indicate that the amount of staining increases. Percent dead copepods were significantly higher inside the motor (dark bars) ($p < 0.05$), whereas they stayed consistently low in the net (light bars) (staining method).

THE PROTOCOL:



ONGOING INVESTIGATIONS:

- (1) Testing the effectiveness of the Neutral Red method for other zooplankton groups.
- (2) Testing the effectiveness of the Neutral Red method under different environmental conditions.
- (3) Monthly sampling of live/dead copepods in Chesapeake Bay.

THE ANILINE BLUE METHOD:

The Neutral Red method does not work well in freshwater environment. We are presently testing another method, the Aniline Blue staining method, for freshwater zooplankton; Dead zooplankton are stained blue, whereas live zooplankton remain unstained:



Live copepod remained unstained. Live elateron is stained in the gut, but dye is ingested food.

Dead copepod and elateron are stained blue.

The Neutral Red and Aniline Blue methods are simple, safe and inexpensive, and can be easily incorporated into routine sampling programs. We are seeking researchers interested in collaboration. Our goal is to establish a global data set on live/dead zooplankton abundance and distribution, both marine and freshwater. If you are interested, please contact:

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ACKNOWLEDGMENTS:

VIMS-OTR program
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REFERENCES:
 Crowder, R. B. 1972. Ches. Sci. 15: 155-159
 Crowder, R. B. 1973. Ches. Sci. 16: 155-159
 Crowder, R. B. 1974. Ches. Sci. 17: 155-159
 Crowder, R. B. 1975. Ches. Sci. 18: 155-159
 Crowder, R. B. 1976. Ches. Sci. 19: 155-159
 Crowder, R. B. 1977. Ches. Sci. 20: 155-159
 Crowder, R. B. 1978. Ches. Sci. 21: 155-159
 Crowder, R. B. 1979. Ches. Sci. 22: 155-159
 Crowder, R. B. 1980. Ches. Sci. 23: 155-159
 Crowder, R. B. 1981. Ches. Sci. 24: 155-159
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 Crowder, R. B. 1984. Ches. Sci. 27: 155-159
 Crowder, R. B. 1985. Ches. Sci. 28: 155-159
 Crowder, R. B. 1986. Ches. Sci. 29: 155-159
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 Crowder, R. B. 2001. Ches. Sci. 44: 155-159
 Crowder, R. B. 2002. Ches. Sci. 45: 155-159
 Crowder, R. B. 2003. Ches. Sci. 46: 155-159
 Crowder, R. B. 2004. Ches. Sci. 47: 155-159
 Crowder, R. B. 2005. Ches. Sci. 48: 155-159
 Crowder, R. B. 2006. Ches. Sci. 49: 155-159
 Crowder, R. B. 2007. Ches. Sci. 50: 155-159