Zooplankton avoidance of a profiled open-path fluorometer

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Significant avoidance of acoustically detected zooplankton was observed in response to a profiling instrument package. Avoidance decreased acoustic scattering from zooplankton averaged over the entire profile by more than a factor of 2, while the maximum avoidance decreased zooplankton acoustic scattering by a factor of 15 over the depth of some discrete scattering layers. Experimental manipulation of the profiler and its instruments revealed that an open-path fluorometer was triggering the avoidance. Avoidance occurred at an average of 8 m below the profiler with a range between 2 and 13 m. Effect range was positively correlated with the average attenuation coefficient of light over the effect range and consistently resulted in avoidance when light levels of approximately 0.013 μmol photons $m^{-2}\,s^{-1}$ were received by the zooplankton. These results have important implications for the analysis of zooplankton data collected from platforms carrying open-path fluorometers and may also warrant careful interpretation of optical measurements from these packages.

KEYWORDS: sensors; ocean optics; ocean acoustics; zooplankton; avoidance

INTRODUCTION

Active avoidance of research vessels and instruments by organisms presents an ongoing challenge in oceanography. Avoidance of instruments can be manifested as an under-estimate of biomass as animals move out of the area or exhibit a change of behavior such as a change in orientation that alters the ability of the instrument to measure the organism or its habitat. Fish avoidance behavior has been observed in response to sampling ships (Olsen, 1990; Soria *et al.*, 1996; Drastik and Kubecka, 2005), trawl nets (Koslow *et al.*, 1995), profiling systems (Graves, 1975; Farmer *et al.*, 1987; Koslow *et al.*, 1995) and camera lights (Benoit-Bird and Au, 2003; Raymond and Widder, 2005). Zooplankton have been shown to avoid nets (Wiebe and Benfield, 2003), optical instruments (Hopcroft, 2001) and profiling packages

(Orr, 1981). Avoidance can substantially affect the measurements of biomass, animal size, species composition and behavior (Ianson *et al.*, 2004).

Avoidance is typically considered only for the target group to be sampled. For example, those using zooplankton net tows consider the avoidance by zooplankton, for instance. Much effort is exerted in the design of most instruments to reduce active avoidance by the target animal. However, some work has shown that avoidance by an animal can affect unrelated measurements of interest. For example, physical microstructure measurements in one study were affected by fish swimming to avoid a profiling instrument (Farmer *et al.*, 1987).

Measurements of incidental avoidance are relatively rare, likely because of the difficulties in measuring avoidance in any setting. Estimating the effects of

avoidance requires a second measurement form that does not induce behavioral avoidance. Often, there are limited alternatives for the measurement approach in question and alternatives are also known to cause some form of avoidance (Brander and Thompson, 1989). In the case of incidental avoidance, there may often be no tool in the study that could measure avoidance by a non-target species.

While active avoidance of oceanographic instruments is difficult to estimate, its causes can be even more difficult to ascertain. Controlled changes in instrument design can reveal the cause of the avoidance in some cases (e.g. Benoit-Bird and Au, 2003; Wiebe *et al.*, 2004), but more often, the specific mechanism of avoidance is inferred. In this study, nighttime avoidance of zooplankton in response to a profiling package was observed and quantified using shipboard bioacoustics. In addition, we experimentally manipulated the profiler and identified the source of the avoidance as a commonly employed open-path fluorometer.

METHOD

From 19 May to 28 May 2008, nighttime sampling was conducted from the R/V *Shana Rae* in the northeast corner of Monterey Bay, California in an area roughly bounded by 36.955°N 121.954°W, 36.933°N 121.896°W, 36.906°N 121.912°W, 36.920°N 121.958°W, in waters between 15 and 35 m deep. Sampling included shipboard, multi-frequency acoustics and vertical profiles with a CTD package that was lowered from a winch ~2 m aft of the echosounder transducers. The purpose of the study was to examine associated layering of phytoplankton and zooplankton.

The transducers of 38, 70, 120 and 200 kHz split-beam echosounders (Simrad EK60s) were mounted 1 m beneath the surface on a rigid pole off the side of the vessel. The 38 kHz transducer had a 12° conical beam. The 70, 120 and 200 kHz transducers each had a 7° conical beam. All four frequencies used a 256 μs pulse resulting in a vertical resolution of 20 cm. Echosounders were calibrated in the field using an indirect procedure incorporating a 38.1 mm diameter tungsten carbide reference sphere as prescribed by Foote *et al*. (Foote *et al.*, 1987).

The profiling package consisted of an SBE19plus CTD with an SBE 43 dissolved oxygen senor, a WetLabs ECO-flntu fluorometer and a WetLabs C-Star transmissometer (25 cm pathlength and 530 nm wavelength). The fluorometer, which uses a 470 nm signal for excitation of fluorescence and a 700 nm signal for turbidity measurement, was pointed directly downward near the leading edge of the profiler's cage.

The acoustic instruments and CTD package were used together during a series of repeated CTD casts conducted every 30 min with intermittent net tows which were integrated vertically from as close to the seafloor as possible, typically 2 m from the bottom, to the surface. A total of 48 net tows were conducted using a 0.75 m diameter, 333 μm mesh ring net equipped with a General Oceanics flow meter. The clocks of all instruments were synchronized at the start of each sampling night and checked for drift at the end of each sampling night. A total of 50, three-cast CTD profiles with all instruments functioning normally were conducted over the course of the experiment.

During several nights, additional profiles were conducted to experiment with the effects of various components of the CTD profiling package. The CTD was profiled in sets of three casts 10 times for each experimental condition: profiler powered off, fluorometer and transmissometer both covered with opaque caps, fluorometer covered, and transmissometer covered. This allowed for exploration of the possible causes of observed changes in the acoustic scattering as a result of the CTD casts.

Decreases in acoustic scattering during CTD profiles were clearly detected at 200 kHz. Reductions in scattering at 120 kHz were substantially weaker and no changes in scattering were detected at lower frequencies. This frequency response suggests that avoidance was occurring within a specific, relatively small size class of scatterers rather than all organisms in the water column. Acoustic scattering at 200 kHz from 2 min, the approximate duration of the first downcast, starting at the time of first cast in each 3-cast set was compared with the acoustic scattering from the 2 min just prior to the profile set. In all cases, the boat was stationary for the acoustic measurements. After removal of the echoes from the CTD package itself which were always visible at depths >5 m, scattering was threshold at −85 dB and integrated from 5 m below the surface, the depth at which CTD avoidance could first be detected, to 1 m above the seafloor to provide a water column integrated index of relative zooplankton abundance. Scattering, measured in linear units of $m^2 \ nmi^{-2}$, was compared using a series of paired *t*-tests, one for each treatment (normal operation, profiler powered off, fluorometer and transmissometer covered, transmissometer covered and fluorometer covered). Analysis of variance (ANOVA) was used to test for treatment effects on the difference between the scattering measured during the profile and that measured just prior to the profile.

In 42 of the 50 "normal" CTD profiles, a well-defined acoustic scattering layer was observed at

200 kHz so that the characteristics of the layer could be defined and measured both before and during CTD profiles. In addition to measuring the change in scattering integrated over the depth of this layer, the vertical offset between the depth of the first detectable change in the depth or intensity of the scattering layer and the depth of the CTD was also measured for each of these profiles to provide an effect range. The percentage of light that would penetrate from the position of the CTD to the position of this first observed effect was estimated using attenuation coefficient data from the 530 nm transmissometer. This was combined with the source light level of the fluorometer at 470 nm in order to estimate the light level received by the acoustic scatterers when avoidance was first observed.

The light emission of the fluorometer was measured using a Satlantic OCR-7 multispectral irradiance sensor. The fluorometer was placed in a 15 cm reflective tube with the multispectral sensor facing towards it on the other end with both ends sealed. As the red light from the scattering sensor was <2% of the total light emission, was highly attenuated in seawater and was likely not to be detected by zooplankton (Raymond and Widder, 2005; see discussion below), the irradiance sensors at 680 nm and greater were covered. Replicate measures of irradiance were made for use in this study.

RESULTS AND DISCUSSION

Evidence of significant avoidance of the CTD profiler was detectable in the acoustic scattering as shown in Fig. 1. The scattering features that showed avoidance were detectable most strongly at 200 kHz with weaker scattering at 120 kHz and nearly no detectable scattering at the lower frequencies. The frequency response of scatterers avoiding the profiler is consistent with the frequency response predicted from small fluid-like zooplankton such as copepods (Stanton *et al.*, 1998). More than 90% of zooplankton in net tows both numerically and by biomass were copepods with a mean size of ~1 mm. The remainder of zooplankton in net samples were relatively soft-bodied organisms including small amphipods, larval euphausiids, chaetognaths, appendicularians and the eggs of a variety of groups. The relatively limited diversity of body types and the lack of any strong scatterers such as gastropods, or those with air inclusions suggest that we can reasonably use 200 kHz scattering as an estimate of relative abundance of these relatively small organisms. An echo-energy integration analysis of the data using the volume scattering at 200 kHz averaged from 2 m above the bottom (the maximum depth of the net tows) to the surface from all

pre-cast samples combined with an estimated target strength at 200 kHz of -92 dB for the averaged length copepod from net tows (Macaulay *et al.*, 1995; Stanton *et al.*, 1998) provides an estimate of the water column averaged copepod density of 63 individuals/m^3. This compares well with the mean density of copepods from net tows of 51 individuals/m^3, further supporting the conclusion that the primary small scatterers in the layers identified at 200 kHz were copepods rather than larger animals that would have avoided the net.

The avoidance of the profiler by these zooplankton resulted in a substantial decrease in the acoustic scattering in the volume beneath the CTD. On average, the total integrated scattering decreased by about half over the entire duration of a cast. At the peak of avoidance, the total integrated scattering decreased by a factor of four. This avoidance occurred rapidly but acoustic scattering returned to near pre-cast levels in time for avoidance to be observed on a subsequent cast (Fig. 2). An ANOVA showed that there was a significant effect of treatment (e.g. normal CTD, fluorometer covered etc.) on the difference in acoustic scattering prior to and during the CTD profile ($F = 19.96$; df $= 3$, 86; $P < 0.01$). A series of paired *t*-tests (Table I) showed that the primary cause of the avoidance of zooplankton was the fluorometer and not the profiling package or the transmissometer. However, not all acoustic scatterers avoided the fluorometer with equal strength. For example, the deep layer in Fig. 1 shows a much greater decrease in acoustic scattering than the midwater layer. These layers have different frequency responses in their volume backscatter, suggesting that are made up of different species or size classes, accounting for differences in their behavioral responses to the instrument package. Such compositional differences would not be measured with the net tows used and thus these behavioral differences cannot be examined in further detail here.

Avoidance detected just within the depth of clearly defined 200 kHz scattering layers observed in 42 of 50 "normal" profiles resulted in an average decrease in scattering within the layer by a factor of 15 relative to pre-cast acoustic scattering. Avoidance effects in these distinct layers were detected when the CTD package was $2-13$ m above the 200 kHz scattering layer with a mean distance between the CTD and the layer of 8.2 m. The effect distance was positively related to the mean beam attenuation coefficient at 530 nm (Fig. 3A, $R^2 = 0.70$, $P < 0.01$). In clearer water where light penetrates further, zooplankton avoided the CTD from further away, while in water less penetrable to light, zooplankton avoided the CTD only when it was close. The percent of fluorometer's light estimated to reach the depth of the zooplankton remained relatively constant
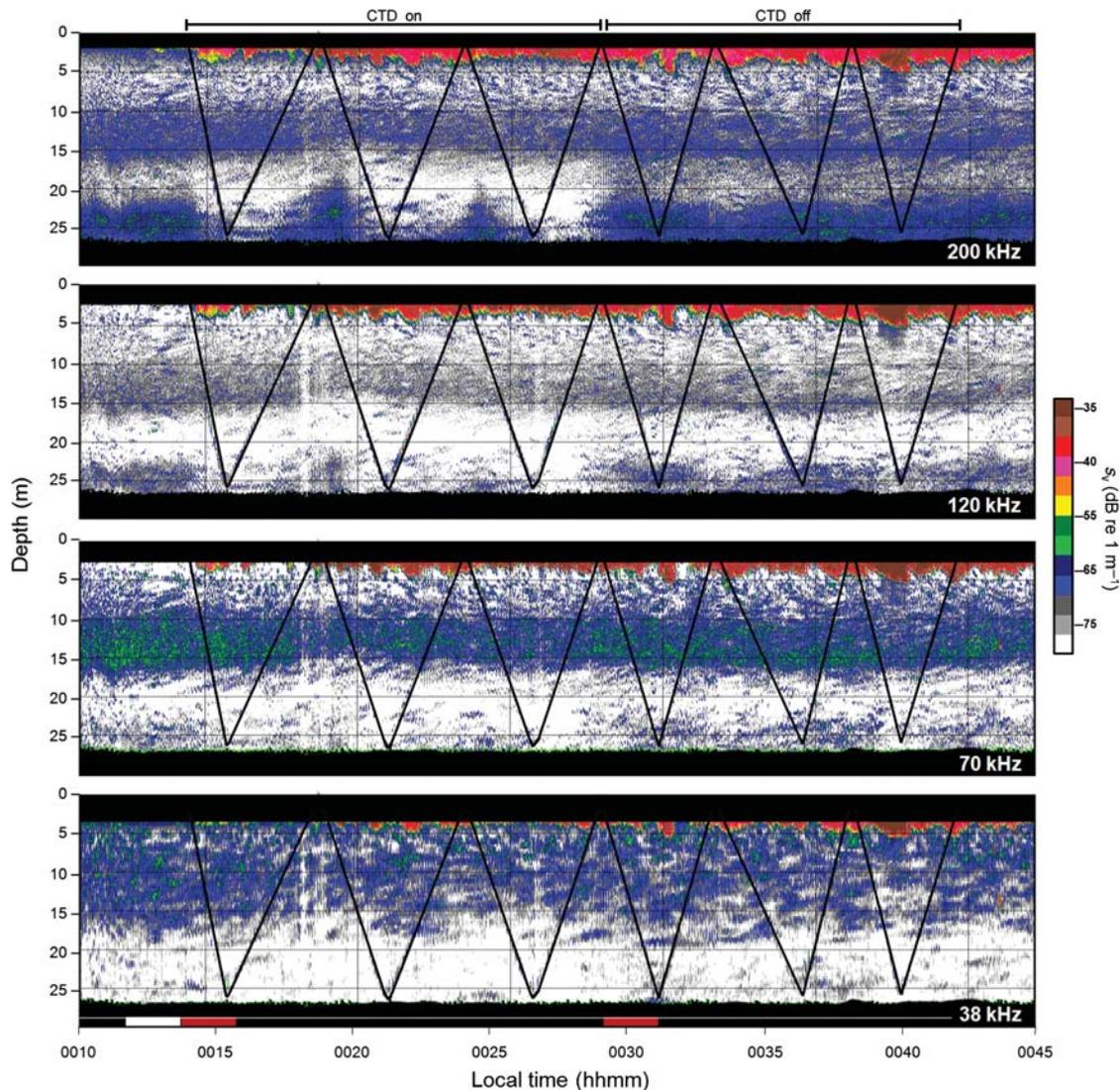
Fig. 1. Sample echograms at four frequencies overlaid with the depth profiles of six CTD casts, three normal and three with the CTD powered off. Acoustic volume backscattering in dB is shown in color with white representing scattering below threshold. The strong scattering near the surface is from air bubbles entrained by the boat's hull. Noticeable decreases in acoustic scattering a few meters above the seafloor (shown in black) can be seen in the 200 kHz echogram (top panel) with somewhat weaker decreases midwater can be seen when the CTD was powered on, but no avoidance is detectable when the CTD was not powered indicating that the avoidance was not caused by the physical movement of the profiling package. Avoidance is less apparent at 120 kHz and undetectable at lower frequencies. The 2-min time interval integrated to provide a background estimate of acoustic scattering is indicated by a white bar near the time axis, while the acoustic integration intervals during the first cast of each profile set are indicated by red bars.

at between 0.005 and 0.075% of source light level with no significant relationship between effect distance and percent of source light received by zooplankton ($R^2 = 0.06$, $P > 0.05$). The light emitted from the fluorometer was measured at 57.92 ± 2.13 µmol photons $m^{-2} s^{-1}$, broadly covering the spectrum from 430 and 500 nm with a peak at 470 nm. Combining this estimate of light emitted by the fluorometer with the light transmission measurements provides an estimate of the light received by acoustic scatterers when avoidance was first detected.

There is no significant relationship between detection range and received light level (Fig. 3B, $R^2 = 0.06$, $P > 0.05$). The received light level was relatively constant, between 0.003 and 0.030 µmol photons $m^{-2} s^{-1}$ with a mean received light level for avoidance of 0.013 µmol photons $m^{-2} s^{-1}$. This supports the conclusion that the observed avoidance of the CTD package is caused by sensitivity to the light from the fluorometer and provides an estimate of the sensitivity of these animals to the fluorometer's light source. This
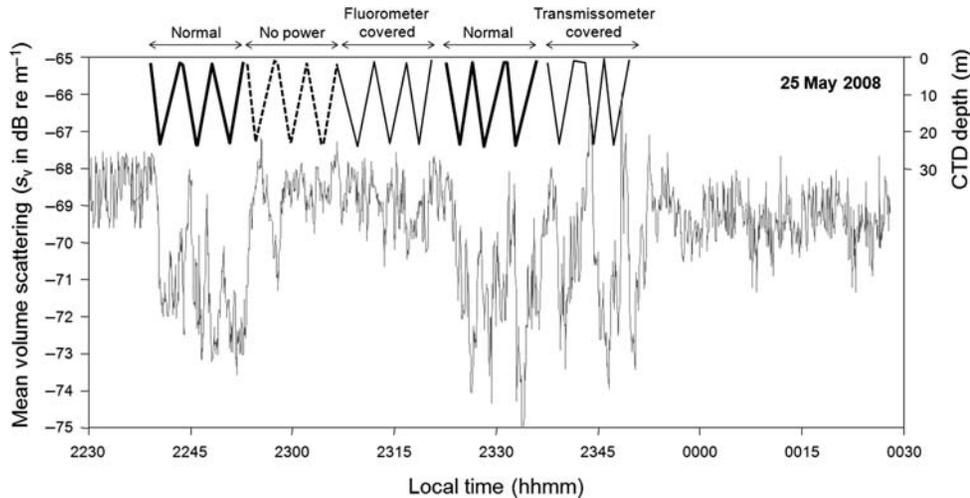
Fig. 2. 200 kHz volume backscattering strength in dB, a log-scaled value, averaged in the linear domain from 5 m below the surface to 1 m above the bottom as a function of time. Note that echoes from the CTD were removed before analysis. The depth profiles of CTD casts are shown across the top. CTD profiles were taken in sets of three repetitive casts before the state of the CTD was manipulated and the profiles repeated. The mean volume backscattering changed dramatically when the CTD was profiled normally or when only the transmissometer was covered with opaque caps. However, when the CTD was turned off or the fluorometer was capped, the volume scattering strength remained similar to the pre- and post-cast values.

Table I: Summary of paired t-test results for the effects of CTD profiling on integrated acoustic backscattering (NASC) for normal profiling and for four experimental treatments used to determine the cause of the observed avoidance

	Mean NASC prior to cast−NASC during cast	t	df	P-value
Normal profiles	36.33	5.56	49	<0.001
CTD powered off	−2.39	1.08	9	ns
Fluorometer and transmissometer covered	3.45	1.82	9	ns
Fluorometer covered	1.72	0.74	9	ns
Transmissometer covered	41.88	4.97	9	<0.01

"ns" indicates results that were not significant at the $P = 0.05$ level.

is consistent with laboratory studies of the wavelengths of light copepods are sensitive to. While the spectral response of copepods varies between species, the light emitted by the fluorometer is within the range of peak sensitivity for many copepod species and is similar to the spectrum of light found during twilight periods, a range of wavelengths which many vertically migrating species are particularly responsive to (Stearns and Forward, 1984; Cohen and Forward, 2002). Our estimates of the level at which a response could be detected are well above the absolute sensitivity limits of at least some copepod species at these wavelengths (Stearns and Forward, 1984; Cronin, 1986). It is important to

recognize that the received light levels shown are only estimates because of the small offset in the wavelength of the light used by the fluorometer and that used to measure light transmission.

Despite the fact that the offset between the fluorometer's excitation wavelength and the transmissometer's wavelength are relatively small and many copepods are relatively equally sensitive to light across this range (Cohen and Forward, 2002), our experimental results show no avoidance of the 530 nm transmissometer. Interestingly, the color of the excitation light from the fluorometer is within 10 nm of the peak wavelength of dinoflagellate bioluminescence (Moline *et al.*, 2007). A number of studies have demonstrated that dinoflagellate bioluminescence plays a role in trophic dynamics by providing predators (i.e. fishes and cephalopods) visual cues for potential copepod prey (e.g. Fleisher and Case, 1995). From the perspective of the copepod, however, this attraction of predators is extremely costly, as evidenced by strong defensive photophobic responses by copepods to even weak simulated bioluminescent flashes (Buskey and Swift, 1985). This is consistent with the copepod avoidance measured in this study and provides a potential behavioral mechanism for interpreting our observations.

CONCLUSIONS

We observed zooplankton avoidance to a CTD profiling package resulting in a factor of two decrease in 200 kHz acoustic scattering averaged over the entire CTD profile

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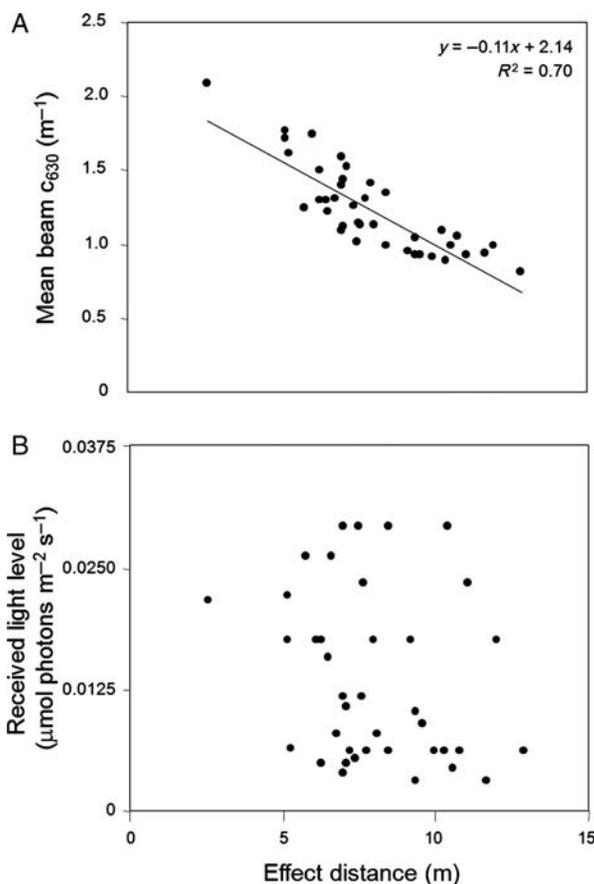
Fig. 3. (**A**) Mean attenuation coefficient measured at 530 nm between the CTD and the depth of the first detectable avoidance of acoustic scatters as a function of the range between the CTD and the avoiding zooplankton. There is a significant, positive relationship between the two variables. As water clarity increased, so did the range at which zooplankton exhibited avoidance of the CTD. (**B**) Light from the fluorometer estimated to be received by the zooplankton when avoidance was first detected as a function of the distance between the CTD package and the zooplankton. There is no significant correlation between the two variables and the range of light levels is quite small, suggesting that the response is driven by the presence of a fixed level of light.

and a factor of four decrease in integrated acoustic scattering at the peak of the avoidance which typically occurred just after the profiler reached the bottom of each cast. Maximum decreases of 15-fold in scattering within distinct scattering layers were sometimes detected. These 4- to 15-fold decreases in zooplankton scattering and likely biomass are what a short-range zooplankton sensor on the profiling package would experience, resulting in highly inaccurate measurements of zooplankton from the profiler.

The zooplankton avoidance observed was not caused by the movement of the profiler itself or by any electrical signals. Covering the transmissometer did not reduce the observed avoidance either; however, covering the open-path fluorometer eliminated any measureable zooplankton avoidance. Avoidance responses of zooplankton were observed up to 13 m away from the CTD package, about one-third to one half of the total water column depth in this study. Orr (1981) observed similar avoidance distances, up to 15 m, by zooplankton to a profiling package. However, he was unable to determine the mechanism of avoidance. Measurements of the optical properties of the water column here showed that avoidance range was positively related to the beam attenuation coefficient so that the light level from the fluorometer received by the zooplankton at the onset of avoidance remained relatively constant at approximately $0.013 \, \mu$mol photons m^{-2} s^{-1} (Fig. 3).

These results highlight the caution that needs to be taken when interpreting zooplankton data from *in situ* instrument packages, specifically those carrying increasingly popular open-path fluorometers. Deployment of these fluorometers in or near the mouths of zooplankton nets warrants special concern as light sources have been demonstrated to affect catch, sometimes even increasing catch efficiency (Wiebe *et al.*, 2004). Care may also be justified when interpreting phytoplankton measures. While it was not possible to independently assess the influence on phytoplankton, the assemblage at the study site was made up of large numbers of dinoflagellates (Moline *et al.*, 2008) that exhibit diel migrations (Schofield *et al.*, 2006) and are thus likely to be sensitive to changes in light. While not capable of rapid movement, even moderate displacement and/or change in orientation of phytoplankton within the 3 cm sampling range of the instrument may influence the resulting fluorescence and other optical signals. Other incidental effects of avoidance such as those detected by Farmer *et al.* (1987) in measurements of physical processes cannot be ruled out. In order to minimize the potential of the problem of avoidance, these results lead us to recommend the use of flow-through rather than open-path fluorometers when low power and small size are not critical, particularly when zooplankton are being sampled concurrently.

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