RESEARCH ARTICLE

Measuring Body Size in Small Marine Fishes: A Comparison of Three Non-intrusive Methods

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Measuring Body Size in Small Marine Fishes: A Comparison of Three Non-intrusive Methods

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Abstract
Studies of non-intrusive techniques are important in fisheries biology, because research methods may inadvertently cause damage to the study organisms. In addition, current effects of human–environment interactions coupled with future trends in global climate change likely will lead to increased monitoring of fish population dynamics. The aim of this study is to analyze the effectiveness of three simple non-intrusive techniques to accurately obtain body length measurements of anemonefish and other small fishes. Frequently used catch and re-capture methods are stressful to fishes, and can alter their behaviors upon release, thus negatively impacting field ecological studies. Alternate methods to non-intrusive sizing of reef fishes are needed, and these methods should be compared to determine the most effective and efficient means of collecting the targeted data. Three non-intrusive techniques were employed to obtain accurate fork length (FL) measurements of the two-band anemonefish, *Amphiprion bicinctus.* Comparison of these methods revealed that fish lengths from visual estimates by self-contained under water breathing apparatus (SCUBA) divers did not differ significantly from those estimated using both video-mirror and Tps-mirror techniques (ANOVA, $F(2,60) = 1.572$; $p = 0.22$). Under laboratory conditions, fish sizes from manual measurements also did not differ significantly from those obtained using either mirror method (ANOVA, $F(2,81) = 0.489$; $p = 0.61$), demonstrating that the mirror techniques accurately assess fish size under both laboratory and field conditions. These methods were not effective in identifying or tracking individual fish among years in the field, due to high rates of fish mobility and turnover. However, they were useful in determining short-term anemonefish migration among sea anemone hosts.

Keywords: Biodiversity; obligate symbiosis; population dynamics; fish body size; anemonefish; giant sea anemone.

1. Introduction

Body length measurements are important for determining the growth rates and population size structure of fishes. In fish populations that experience stable recruitment and mortality [1], body size frequencies can also be applied to the Beverton–Holt model to calculate productivity and population yield for the sustainable management of fisheries [2]. This model was used to characterize not only the population dynamics of fishes, but also of many other marine organisms, including some stony corals [3]. Data from the Beverton–Holt fishery model also can be fitted to von Bertalanffy growth curves [4] to estimate age–size relationships in fishes and other organisms.

Common techniques used to acquire fish body size measurements, such as catch and release, hook and line, electro-fishing and anesthetics can cause physical damage and physiological stress to the fish [5–7]. Although these intrusive methods are often used to collect fish length data, they may alter subsequent fish behavior during long-term field studies. Reduction of fish stress therefore requires sizing methods that rely on observation from a distance, but the non-intrusive methods employed to date had limited success. Brock initially used visual census to assess fish body sizes on coral reefs [8], but it was suggested that it was difficult to obtain accurate fish lengths by visual estimation underwater [9]. Furthermore, problems were reported with observations at a distance using an underwater auto-focus video camera mounted on a Remotely Operated Vehicle [10]. Consequently, laser-tagging was used to collect fish measurements, which proved to be a more accurate but much more expensive method.

The use of video cameras in conjunction with mirrors may allow accurate determination of live fish lengths, because many fishes are attracted to their mirror images, and even display parallel swimming with their images, causing them to line up closely with length markings on the mirror surface [8]. This video-mirror method also provides a visual record of fish appearance, thus potentially allowing long-term identification of individuals. This method has been applied far only to assess measurement efficiency, in terms of the number of video clips required to obtain length measurements for each fish during a single self-contained under water breathing apparatus (SCUBA) dive [8].

Little is known about the long-term growth rates of anemonefishes in the field, in part because these fish are negatively impacted by standard catch and re-capture methods [11–13], hence there is a need to develop a non-intrusive method to identify them and measure their body sizes. The accuracy of the video-mirror technique can be tested easily in laboratory aquaria, where the fish are accustomed to handling and thus less negatively impacting by manual measurements of body size.

In the Red Sea, endemic two-band anemonefish *Amphiprion bicinctus* are obligate mutualists with three species of giant sea anemone hosts: *Entacmaea quadricolor*, *Heteractis crispa* and *Heteractis magnifica* [12–14]. These soft-bodied sea anemones provide a unique habitat for anemonefishes, which are protected from piscivorous fishes by the anemones' nematocysts. Furthermore, host anemones benefit from the presence of anemonefishes as they are aggressive against specialized anemone predators such as chaetodontids, and attack them more than they do non-predatory fishes in close vicinity [15]. Recent research has revealed physiological benefits from anemonefish to host anemones in the form of transferred nutrients [16–18] and enhanced gas exchange [19].

The abundance of *A. bicinctus* is highest in Jordan at the northern tip of the Gulf of Aqaba, Red Sea, in comparison with the central and southern coasts of the Red Sea [20]. The average abundance of *A. bicinctus* per 100 m reef transect is 25.22 in Jordan, followed by 2.77 in Egypt, 3.91 in Saudi Arabia, 0.11 in Yemen and only 1.06 in southern Djibouti reefs on the nearby Gulf of Aden [20]. However, these high frequencies of anemonefish on Jordanian reefs are threatened by recent coastal development.

Over the last 30 years, industrial growth in the Red Sea cities of Eilat and Aqaba has led to increase in commercial port, aquaculture and tourism activities, resulting in rising domestic and industrial effluents such as oil, fertilizers and pesticides on coral reefs along the coasts of Israel [21, 22] and Jordan [23]. These anthropogenic stressors likely impact patterns of sea anemone and anemonefish recruitment, growth and mortality due to alteration of the environmental conditions on nearshore coral reefs and field methods are needed to accurately size the anemonefish and determine these demographic changes. The purpose of the present study was to assess the three methods of measuring small marine fishes including anemonefishes, using inexpensive techniques in the laboratory and the field. Specifically, the usefulness of the video-mirror method was examined as a tool to accurately assess fish body size and identify individuals.

2. Methods

Preliminary trials of video-mirror laboratory experiments were conducted in aquaria [17] at Auburn University in January 2009. Anemonefish that were originally transported to Auburn in 2006 from a culture facility at oceans, reefs and aquariums (ORA, Florida, USA) were observed in laboratory aquaria to which mirrors had been added. These preliminary trials aided in selection of the mirror size to use for later laboratory and field fish measurements, also determined the period of time needed for anemonefish to adjust their aggressive behavior and to begin parallel swimming adjacent to the mirror. In September 2010, a total of 28 anemonefish (16 adults, 12 juveniles) were measured in the laboratory using (a) hand-held calipers (i.e., manually), (b) the video-mirror technique and (c) the Tps-mirror technique. These methods are discussed in detail in the laboratory section (below).

In June 2009, 21 anemonefish on the coral reef adjacent to the Marine Science Station at Aqaba, Jordan (N 29 31′, E 35 0′) were selected. Divers using SCUBA recorded these anemonefish fork lengths (FL) using visual estimates, and the video-mirror and Tps-mirror techniques. These measurements were used to compare these three non-intrusive techniques in the field.

2.1. Laboratory measurements

Fish body size measurements were made under laboratory conditions on 16 adult (FL $>$ 60.1 mm, [24]) two-band anemonefish *A. bicinctus* (FL $= 113.7 \pm 12.0$ mm, mean $\pm$ sd) and 12 juveniles (FL $<$ 60 mm, 59.0 ± 13.7 mm, for details of culture conditions see [17]). To obtain video-mirror measurements of fish body size, a 20×20 cm glass mirror bordered by alternating 1 cm orange marks (for scale bars) was placed inside the home aquarium of each fish. Based on preliminary observations, each individual was allowed to acclimate 1 min to the presence of the mirror, and then videotaped for 30–60 s using a digital camera (Samsung Digimax A503). Images from each video sequence later were viewed on a computer screen, and analyzed to obtain fish lengths [8].

In the video sequences, individuals of *A. bicinctus* were observed to display parallel swimming back and forth adjacent to the mirror surface. The video playback speed was slowed during these sequences, and images viewed until one was obtained of the fish positioned parallel and close to the mirror surface. The video was paused at this image, and the video frame number recorded. Hand-held calipers were used to obtain an on-screen FL measurement, followed by a FL measurement using the scale bar markings on the mirror. A correction coefficient was calculated from the ratio of these measurements (scale bar markings = on-screen fish length, after [8]). The actual video-mirror fish length was calculated by multiplying the correction coefficient by the on-screen fish length measurement.

A morphometric computer program TpsDig 2 (http://life.bio.sunysb.edu/morph/) was applied to assess the accuracy of the video-mirror technique, and this modified technique was termed the Tps-mirror method [25]. This software was designed to digitize landmarks and outlines for morphometric analyses, and each selected video frame was stored as an extension file for a top speed (Tps) Database, which is a type of file that saves data entries, one entry at a time. This software was used to analyze the above video frames, as an additional fish body size analysis to compare with the video-mirror method. Each recorded video was opened in the TpsDig 2 software, as the one described above for the video-mirror method was captured, saved and re-opened in the Tps-utility program, where a digital scale allowed for more accurate calculation of fish length measurements.

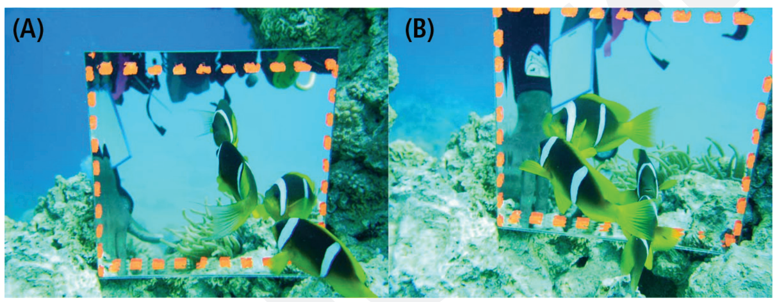
After each fish was videotaped under laboratory conditions, it was removed from its home aquarium using a fine mesh net, transferred to a paper towel, briefly blotted to remove excess water, and its FL measured manually using calipers (tip of snout to posterior end of middle caudal rays, www.fishbase.org). Each fish was out of water for ~30 sec during this manual measurement of body size, and all fish appeared to swim normally within a few minutes after return to their home aquaria. These manual FL measurements provided the exact body length of each fish, and were compared to the other two methods above.

2.2. Field measurements

During June 2009, the body sizes of two-band anemonefish *A. bicinctus* on a coral reef adjacent to the Marine Science Station at Aqaba, Jordan (N 29 31′, E 35 0′) was measured. SCUBA divers visually estimated the FL of each anemonefish at the study site (N $=$ 112), using scale bars marked in cm on their underwater data slates. Divers carefully extended their slates as close to each fish as possible, then visually estimated FL, rounding to the nearest 0.5–1.0 cm. During these visual estimations, each dive slate with a scale bar was held $>$10 cm from each measured fish, because even though the fish did not desert their host sea anemones during measurements, they actively avoided the dive slates.

Of the 112 fish measured by visual estimation, half (56) were selected randomly for video-mirror assessment, due to limited time underwater for videotaping. Preliminary observations underwater further reduced this number to 21 fish that were logistically the easiest to record on videotape, due to the orientations of their sea anemones on the coral reef, lack of obstructing nearby reef structures, and fish behavior in relation to the mirror surface. A marked mirror (Figure 1A and 1B) was placed adjacent to the sea anemone host of each selected fish, then the diver (in all cases S.G. Belford) moved to a distance of 0.75–1.0 m from the sea anemone. Fish were allowed to acclimate to the mirror for 30 s, then videoed for 60 s using a Sea & Sea DX-860G digital camera and underwater housing. In most cases, images of each fish swimming parallel and close to the mirror were observed during this initial 60 s video period; if not, an additional 60 s was recorded. Fish fork lengths from video sequences obtained under field conditions then were analyzed and compared to those obtained using the other methods described above (field visual estimation and the three laboratory measurement methods).

Figure 1: Video-mirror images for the analysis of body size (FL) in the anemonefish *A. bicinctus,* shown here with the giant sea anemone *E. quadricolor* on a coral reef at Aqaba, Jordan during June 2009. (A) Two fish oriented obliquely to the mirror during the 30 s acclimation period. (B) One fish beginning to parallel-swim adjacent to the surface of the mirror, near the start of 60 s of video recording. Note that the 1 cm scale marks surrounding the edges of the mirror are clearly visible in the video images.



3. Results

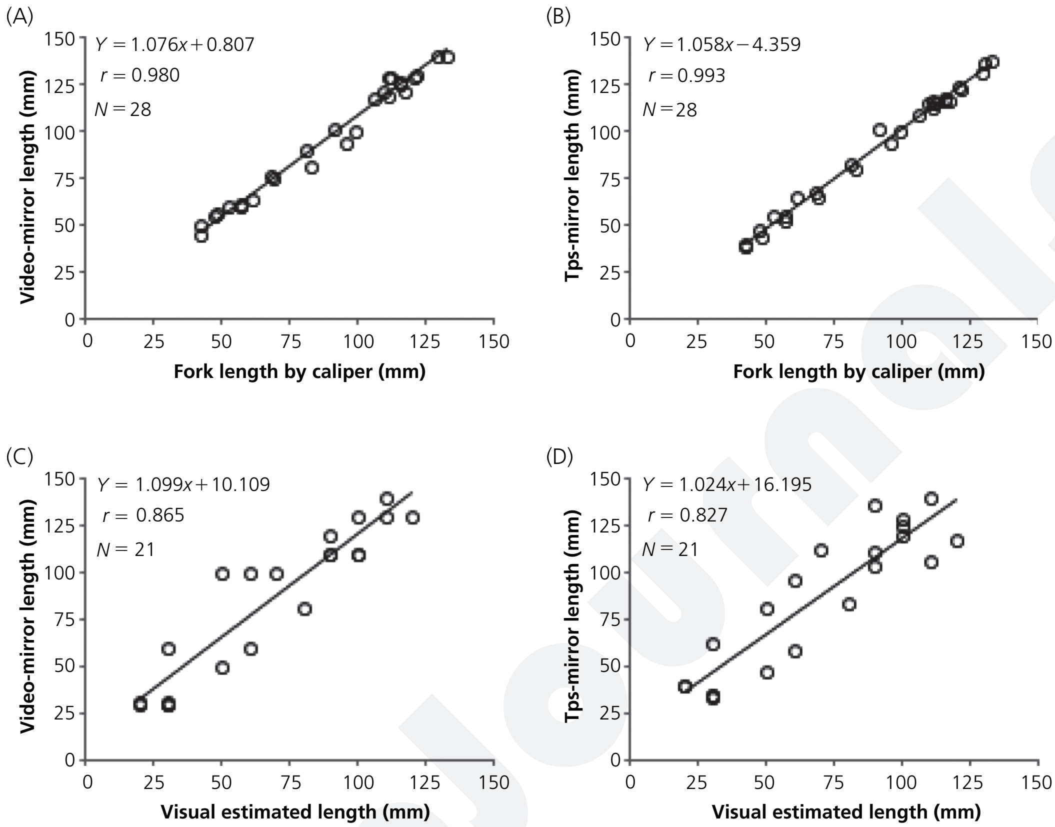
3.1. Laboratory measurements

Anemonefish FL did not differ significantly among the three laboratory measurement methods (manual, video-mirror and Tps-mirror (ANOVA, $F(2,81) = 0.489$; $p = 0.61$)). Manual measurements using calipers were slightly but not significantly smaller (113.7 ± 12 mm for adults; 59.1 ± 13.7 mm for juveniles) than those using both the video-mirror (123.2 ± 15.4 mm for adults; 64.4 ± 13.4 mm for juveniles) and Tps-mirror methods (116.2 ± 12.2 mm for adults; 57.8 ± 14.4 mm for juveniles). Manual lengths correlated tightly with those obtained from both video-mirror ($r = 0.980$) and Tps-mirror methods ($r = 0.993$, Figure 2A and 2B). Of the two non-intrusive fish sizing methods, the Tps-mirror method was the most efficient as required much less time than the video-mirror method, which required both on-screen and reference measurements, and then calculation of a correction coefficient. Additionally, the Tps-mirror method did not require fish removal from aquaria, nor did it cause fish to increase their respiratory activity, which usually results from stressful situations.

3.2. Field measurements

Anemonefish FL did not differ significantly among the three field measurement methods tested (visual estimation, video-mirror and Tps-mirror (ANOVA, $F(2,60) = 1.572$; $p = 0.22$)). Fish body lengths estimated visually by SCUBA divers were shorter than those obtained by both video-mirror and Tps-mirror methods, which did not differ significantly from each other in the fish lengths obtained. The fish lengths estimated visually underwater correlated with those obtained by both video-mirror ($r = 0.865$) and Tps-mirror methods ($r = 0.827$) in the field, but these correlations were much looser than those between fish measurements obtained manually versus with mirrors under laboratory conditions (Figure 2C and 2D).

Figure 2: Covariation in FL of the two-band anemonefish *A. bicinctus* obtained using various measurement techniques under laboratory and field conditions. (A) Video-mirror versus manual (caliper) method in the laboratory, (B) Tps-mirror versus manual (caliper) method in the laboratory, (C) Video-mirror versus visual estimation method in the field and (D) Tps-mirror versus visual estimation method in the field.



4. Discussion

Results show that non-intrusive methods which measure fish lengths using video cameras and mirrors are more accurate than that visual estimation by SCUBA divers in field studies on coral reef fish. However, divers' visual estimations can also be a reliable source of fish size data. In addition, photographic identification in the field can serve as a method to track species at a given location. Of the two mirror methods examined, the Tps-mirror technique is less time consuming than the video-mirror method under both laboratory and field conditions. The TpsDig 2 geometry morphometric software employed in the Tps-mirror method is open access and can be downloaded for free (http://life.bio.sunysb.edu/morph/), adding to its utility for body size analyses.

In terms of the expense of each method, the cost of a small mirror is negligible, and with current technology, digital underwater cameras have also become relatively inexpensive, so these video-mirror methods are not much more expensive than visual estimation for field assessment of fish sizes. For the use of either mirror method, experienced SCUBA divers are needed because good buoyancy control was more important for efficient video collection than for visual estimates using dive slates in the field. Also, video collection requires more time per fish than does visual estimation, so fewer fish can be measured during each SCUBA dive. Thus, the video-mirror method can be used efficiently only by experienced divers with good buoyancy control and adequate time underwater, which may be a limitation in some field studies where inexperienced students or volunteer divers are involved, and/or field time is highly limited.

The territorial behavior of anemonefishes toward their reflections in mirrors causes them to closely approach mirrors and swim parallel to their own images, which greatly assists with video collection [8].

This method was most useful for measuring territorial fishes that closely approach mirrors, and is expected to require more time or even to be unworkable for fishes that are not attracted to their mirror images.

Accurate determination of fish size using visual estimates underwater is difficult, because divers differ in their visual perceptions of fish lengths. Each diver in the present study estimated fish FL at a distance from the fish (~50−75 cm from dive slate to fish). In contrast, a diver can videotape fish that are adjacent to mirrors from any working distance (about 100−150 cm in the present study), as long as the mirror marks are not blurry in the video images, and the fish are not disturbed by the diver presence. Another limitation to visual estimation of fish lengths occurs if fish are incubating eggs. When guarding egg masses adjacent to their sea anemones, both members of anemonefish breeding pairs are extremely territorial and will aggressively attack divers' hands during attempts to measure their body lengths using dive slates (S.G. Belford, personal observation). This is not a problem with the mirror technique, because fish are more attracted to their mirror reflection as a perceived intruder, than to the diver, who is able to stay further away from the anemone (about 100−150 cm distant, see above) than with visual estimation measurements.

SCUBA divers may visually estimate fish lengths non-intrusively if trained to estimate from a distance, but they tend to underestimate body lengths by over-compensating for the 30% increase in the size of objects underwater [10]. Errors decrease as divers become trained to recognize lengths underwater, but then return when trained divers don't dive for 6 months and subsequently attempt to measure fish sizes. The fish lengths were estimated visually underwater and shorter than those measured more accurately using video. This were highly correlated with video measurements, and so remain a viable method for field estimation of fish sizes, as long as the correction factor is taken into account.

Preliminary catch and release trials in the field indicated that anemonefish became extremely agitated when captured for size measurements, and many of them rejected their host anemones upon re-release, and could not be relocated later at the study site (N.E. Chadwick, personal observation). Thus, fish capture for size estimates may interfere with normal behavior and was an unworkable method for studies on the long-term demographics or ecology of some fishes. Some anemonefishes can be distinguished individually by their color patterns and relative body sizes, thus video images potentially can be used for individual identification. Consequently, this method requires frequently revisiting fish in the field to track their movement patterns, because some anemonefish migrate often among host sea anemones ([13], S. Belford, personal observation). Thus, video-identification of anemonefish may be useful over days to months in the field, but does not necessarily allow easy identification among years of study.

Although some success in using the video-mirror technique to track anemonefish migration was achieved, this technique required a large time investment for individual identifications. Thus, related study on anemonefish migration patterns between sea anemone host species (*E. quadricolor* and *H. crispa*), visually estimated fish body sizes and recorded identifying marks (e.g., shapes of white bands on the body) for use in tracking individuals [13].

Demographic data on reef organisms provide baseline information that can assist authorities in monitoring the condition of coral reefs, and thus in managing reef revenue from fishing and tourism [26]. Both the video-mirror and Tps-mirror techniques are simple, effective methods to monitor population size frequencies and also potentially short-term growth in some reef fishes. This demographic information can provide a scientific basis for the sustainable management of ornamental fisheries, especially for anemonefishes which often are under threat due to intensive collection for the marine aquarium trade [27, 28].

5. Conclusion

Current climate patterns require extensive use of field measurements for population and biodiversity monitoring studies on coral reefs. Furthermore, continued coral reef monitoring of the anemonefish mutualism may reveal this symbiosis to be an essential bio-indicator for bleaching events. All these measurement techniques can be used effectively, but especially the mirror techniques have the potential to add accurate fish body size information to future population dynamic studies on coral reefs.

The measurement techniques examined, allowed extensive data to be collected over short periods of time in the field. The aggressive nature of anemonefishes toward any entity approaching their host sea anemones causes them to become physiologically stressed when closedly approached or handled. As such, the primary goals of the mirror measurement techniques examined were to decrease the time spent by divers close to the sea anemone, and to demonstrate that both techniques can be used to accurately size anemonefishes, decreasing their behavioral changes due to unnatural stressors.

Abbreviations

Length: mm, cm, m;
Time in seconds: s;
Analysis of variance: ANOVA;
Standard deviation: sd;
Correlation coefficient: *r*.

Competing Interests

The authors declare that they have no competing interest.

Authors' Contributions

SGB carried out the experiments, collected and analysed data and drafted the manuscript. MK made valuable suggestions for the improvement of the overall manuscript and drafted the manuscript. NEC assisted with the collection and analysis of data and drafted the manuscript.

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