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### Deep-Sea Fish Behavioral Responses to Underwater Vehicles: Differences Among Vehicles, Habitats and Species

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#### 1. Introduction

Fishes have a wide range of perceptual capabilities allowing them to behaviorally respond to various environmental stimuli such as visual, acoustic, mechanical, chemical, and electromagnetic signals. In our "noisy" world of today many artificially evoked signals pass through aquatic habitats, where fishes perceive them and respond to in often unpredictable manner. Proper distinction between natural and artificially evoked (="disturbed") behavior is of utmost importance in ecological studies that try to identify the prevailing factors and mechanisms influencing fish abundance, distribution and diversity.

As we know today, the need to consider human-induced behavioral disturbance as an important factor in ecological studies (Beale 2007) applies even to inhabitants of remote aquatic habitats such as the deep sea. *In situ* studies using various types of underwater vehicles (UV's) have significantly changed the conception that the inhabitants of the deep, dark and mostly cold ocean are less behaviorally active and hence less susceptible to anthropogenic disturbance. While direct observation of deep-sea animals goes back to the time of William Beebe in the 1930s, *in situ* studies of deep ocean organisms and their habitats have become increasingly more common during the last 50 years.

After initial use for exploration and discovery of yet unknown habitats and organisms, UV's were adopted to systematically investigate the ecology of deep-sea organisms, especially the larger and easier observable fauna in the open water and close to the bottoms. In analogy to census studies conducted by divers in shallow waters, vertical or horizontal transects with underwater vehicles were used to obtain density or distributional data of fishes (e.g., Yoklavich et al. 2007, Uiblein et al. 2010). Distinct fish species or closely related taxonomic groups were found to occur at relatively high densities during such transects allowing quantitative behavioral investigations.

Early *in situ* exploration encountered first evidence of pelagic and bottom-associated (demersal) fishes living at depths well below 200 m being behaviorally active similar to shallow-water species (Beebe 1930, Heezen & Hollister 1971). These preliminary behavioral observations were followed by detailed studies of locomotion behavior and habitat utilization based mainly on video equipment employed during bottom transects with manned submersibles (e.g., Lorance et al. 2002, Uiblein et al. 2002, 2003) and later with

ROV's (e.g., Trenkel et al. 2004a, Lorance et al. 2006). Quantitative behavioral comparisons conducted with the submersible Nautile clearly showed that fish species differ among each other in the way they swim and in their vertical positioning above the bottom (Uiblein et al. 2003). Moreover, distinct responses to the approaching vehicle were identified which needed to be analyzed in detail so to be able to distinguish natural behavior from responses to anthropogenic disturbance. That underwater vehicles have a disturbance effect on fish behavior has also important consequences for fish density calculations from *in situ* transects, as the data may not reflect natural conditions when disturbance responses are intense and/or occur frequently (Trenkel et al. 2004b, Stone et al. 2008).

Disturbance responses in deep-sea fishes may be caused by a number of factors like noise produced by motors and thrusters, light used for illumination purposes, motion, electromagnetic fields, or odor plumes deriving from the vehicle. Detailed investigations regarding the actual source(s) of disturbance are generally lacking. Here, a description and categorization of disturbance responses is provided and differences between vehicles, habitats, and species are elaborated. These data suggest that disturbance responses are manifold and can – by themselves – reveal interesting insights into the life modes of deep-sea fishes. In addition, when disturbance responses are identified, natural behavior (e.g., locomotion and vertical positioning above bottom) can be filtered out and studied independently of artificial evocation.

Here, nine case studies based on manned submersible and ROV video transects in the deep North Atlantic are presented dealing subsequently with differences in disturbance responses between underwater vehicles, dive transects (habitats), and co-occurring species/species groups. In addition, a separate section is devoted to combined analyses of natural behavior and disturbance responses, to show the full picture. These results are discussed referring to (1) novel insights about deep-sea fish artificially and naturally aroused behavior, (2) the need for consideration and integration of all influential factors in the behavioral analysis and interpretation, and (3) future technological possibilities and challenges towards optimizing *in-situ* investigations on the behavior and ecology of deep-sea fishes.

#### 2. Material and methods

Video recordings from the areas of the Bay of Biscay and the northern Mid-Atlantic Ridge made during six dives with four different underwater vehicles were studied. The underwater vehicles were as follows (Table 1): the manned submersible Nautile and the ROV Victor 6000 (both at IFREMER, www.ifremer.fr), the ROV Aglantha (IMR, www.imr.no), and the ROV Bathysaurus (ARGUS, www.argus-rs.no). Each dive consisted of one to three horizontal transects close to the bottom which lasted between 10 and 174 minutes and covered various depth ranges between 812 and 1465 m (Table 1). During transects the respective vehicle moved slowly (ca. 0.5 to 1.0 knots on average) above the bottom, mostly in straight lines, sometimes interrupted by short stops.

Each of the 10 total transects crossed a distinct habitat within canyons or deep-sea terraces of the Bay of Biscay (Nautile, Victor 6000) and slopes or valleys of the northern Mid-Atlantic Ridge (Aglantha, Bathysaurus) (Table 1). The Mid-Atlantic Ridge study area was divided in a southern investigation box, close to the Azores, and a northern box situated in the area south and north of the Charlie Gibbs Fracture Zone (Table 1).

226

#### Deep-Sea Fish Behavioral Responses to Underwater Vehicles: Differences Among Vehicles, Habitats and Species

									B			
Cruise	OBSERVHAL	VITAL				MAR-ECO						
Region		Bay of Biscay				Northern Mid-Atlantic Ridge						
Vehicle	Submersible Nautile	ROV Victor 6000				ROV Bathysaurus	ROV Aglantha					
Dive	OB22	V	T1	VT3		ME4-2 (SS 44)	ME10 (SS 60)			ME16 (SS 70)		
Transect	OB22-1	VT1-1	VT1-2	VT3-1	VT3-2	ME4-2-1	ME10-1	ME10-2	ME10-3	ME16-1		
Area	M	Mériadzek Terrace		Belle Isle Canyon		Southern Box	Northern Box, south		Northern Box, north			
Date	17.05.98	23.8.02	24.8.02	31.8.02	1.9.02	12.7.04	19.7.04		26.7.04			
Duration (min)	126	174	111	91	85	50	21	10	19	105		
Depth	1250-990	1454 - 1392	1228 - 1208	1193 - 1159	1465 - 1446	1228-1272	954-848	847-812	842-879	1373-1270		
Roundnose grenadier	29	1	1	19	20	3	7	33	20	20		
Orange roughy	5	2	0	3	0	0	0	73	43	0		
Codling	32	29	37	32	33	3	29	11	31	0		
False boarfish	0	33	0	0	0	41	4	3	1	0		

227

Table 1. Overview of dives, vehicles and video transects, with numbers of encountered fish per transect. Samples analyzed are highlighted. For further explanations see text.

Behaviour	Category							
Disturbanca response	No C		Close		Far		Arriving	
Distuibance response	response dis		stanc	ce dist		ance	disturbed	
Vertical position in water	Close to bottom		Well above		ove	East above bottom		
column			bottom		Fai above bottom			
Locomotion behaviour	Inactive Drif		ting Stat		tion holding		Forward moving	

Table 2. Overview of the behavioral categories studied

The four species/species groups selected for detailed analysis were the roundnose grenadier *Coryphaenoides rupestris* (family Macrouridae; Fig.1), the orange roughy *Hoplostethus atlanticus* (family Trachichthyidae; Fig.1) the false boarfish *Neocyttus helgae* (family Oreosomatidae; Fig.1) and codling (family Moridae). The term "codling" includes the most common *Lepdion eques* (North Atlantic codling; Fig. 1), its congeners *L. guentheri* and *L. schmidti*, and the slender codling *Halagyreus johnssonii*. Identification of species/species groups was based on the size and form of the body, head and fins, and color patterns and distributional data from the respective area deriving from collected material.

The recording of all behaviors started immediately after a fish appeared on the video screen. Four main behaviors, overall activity level, disturbance response, locomotion, and vertical positioning above the bottom, each consisting of two or more categories, were recorded for subsequent statistical analysis (Table 2). Fishes visualized on video with high or increasing swimming speed indicating burst swimming in response to prior disturbance by the submersible ("arriving disturbed") were excluded from further-going behavioral analyses. During the subsequent behavioral recordings, the UV frequently got closer to the fishes, with increasing illumination intensity caused by the front lights. If a disturbance response was observed during this process (i.e. a marked change in activity level and/or locomotion behavior), the recordings of locomotion or vertical body positioning were stopped immediately before the occurrence of this behavioral change. The disturbance response during UV approach was split into two separate categories, depending if it happened still at far distance or at close distance to the UV and mostly within the highest illumination radius.



Fig. 1. Photographs of studied fish species (North Atlantic codling was the most common species of the codling group)

For the analysis of undisturbed natural behavior, four locomotion activity categories were identified: "inactive" (Table 2) (= without any movement), "station holding" (= body stationary with active swimming against current), "drifting" (= movement in lateral or backward direction with or without swimming activity), and "forward movement" (= clear active forward swimming movements). Three categories for vertical body positioning in relation to the bottom surface were determined: "close to bottom" (= positioned at the bottom or at distances of less than one body length above the bottom), "well above bottom" (= distance from bottom exceeds one body length), and "far above bottom" (= distance from bottom exceeds three body lengths).

In order to reduce the number of influential factors comparisons between underwater vehicles and species/species groups were mostly restricted to the same transect or area and comparisons among habitats were restricted to single species. Only samples with 19 or more individuals per species/species group encountered per transect were analyzed to allow statistical comparisons in all instances. For statistical comparisons of categorical data among species/species groups and habitats, *G*-tests of independency were carried out (Sokal & Rohlf 1981).

#### 3. Results

The behavioral data of 501 fishes from the four selected species/species groups were analyzed. Apart from a single exception (codling in dive transect OB22-1) disturbance responses occurred during all transects and in all species/species groups. On average 44 % of all fishes showed disturbance and in 7 of the 15 total observational sets (= species-transect combinations) that were analyzed, more than 50 % of the fish displayed disturbance responses. While pre-arrival disturbance was relatively rare (14 % of all disturbed behavior registered), disturbance responses at far distance occurred most frequently (59 %). The disturbance responses were only rarely directed towards any of the four UV's used. No clear signs of attraction or aggressive responses triggered by the UV's could be observed in any of the four species/species groups.

#### Differences between underwater vehicles (Fig.2)

The codling showed a significant difference (p<0.005) in disturbance responses between two dive transects performed in the same area at the Mériadzek terrace, Bay of Biscay, one with the manned submersible Nautile (transect OB22-1, Table 1) and the other with the ROV Victor 6000 (transect VT-1, Table 1). While no disturbance response was registered during the dive with Nautile, 35 % of the individuals encountered during the ROV transect showed clear signs of disturbance. Among the disturbed fish 23 % showed pre-arrival disturbance, while 54 % responded at far distance and 23 % responded at short distance to the approaching vehicle. Regarding undisturbed natural behavior, no significant differences in both vertical positioning and locomotion behavior were found between the two transects.



Fig. 2. Disturbance responses of codling during a manned submersible transect (left) and a ROV transect (right) in the area of Mériadzek Terrace, Bay of Biscay

#### Differences between dive transects and habitats (Fig. 3)

Orange roughy showed significant differences in disturbance responses (p<0.01; Fig. 3a) between two transects that crossed adjacent habitats at similar depths (812-879 m) during dive ME10 (Table 1) with the ROV Aglantha on the northern Mid-Atlantic Ridge, just south

of the Charlie Gibbs Fracture zone. Each of the three categories of disturbance responses decreased in frequency between the first and the second transect thus indicating less responsiveness. Both vertical positioning and locomotion behavior did not differ significantly between transects.



Fig. 3. Disturbance responses of (a) orange roughy during two subsequent ROV transects in the area of the northern Mid-Atlantic Ridge and (b) codling during two subsequent ROV transects in the area of Mériadzek Terrace, Bay of Biscay

The codling showed a significant decrease in disturbance responses (p<0,005; Fig. 3b) between the first and second transect of ROV dive VT1 (Table 1) on the Mériadzek Terrace, Bay of Biscay. These two transects covered different depth zones (1392-1454 vs. 1208-1228 m), the first (VT1-1) being clearly deeper. Neither vertical positioning nor locomotion behavior differed significantly between the two transects.

#### Differences between co-occurring species/species groups (Fig. 4)

During the manned submersible transect OB22-1 on the Mériadzek terrace, roundnose grenadier differed significantly in disturbance responses (p<0.0001; Fig. 4a) from the codling. The former showed all three categories of disturbance, while the latter showed no disturbance responses at all (see also first case study; Fig. 2). Regarding natural behaviour, no differences in vertical positioning occurred, but roundnose grenadier showed significantly more forward movement and less station holding than codling (p<0.01).

During ROV dive transect VT1-1 the codling and the boarfish differed significantly from each other in disturbance responses (p<0.005; Fig. 4b) with the boarfish showing clearly less disturbed arrival and close-distance responses to the approaching vehicle. At far distance from the ROV, the frequency of disturbance responses was similar in both taxa. In addition, significant differences occurred both in vertical positioning and locomotion behavior which are dealt with at the end of the next section.

#### Variation in natural behavior and disturbance responses

Four different comparative data sets were selected (1) to exemplify situations with disturbance responses occurring at constant or variable rates between transects/habitats or between species/species groups and (2) to analyze in detail the undisturbed, natural vertical



Fig. 4. Disturbance responses of (a) codling and roundnose grenadier during a manned submersible transect and (b) codling and false boarfish during a ROV transect in the area of Mériadzek Terrace, Bay of Biscay

positioning and locomotion behavior of these species/species groups during the same dive transects.

a. Differences only in locomotion behavior (Fig. 5)

The disturbance responses of codling did not differ significantly between two transects (ME10-1, ME10-3, Table 1) of a dive with the ROV Bathysaurus on the Mid Atlantic Ridge (Fig. 5a). There was however a significant difference in locomotion behavior (p<0.05). During the first transect all individuals encountered were active and mostly station holding, while several were inactively sitting on the bottom during the third transect, with less fish station holding. Drifting and forward moving occurred in both transects at rather similar rates. No significant differences in vertical positioning occurred.

During the ROV transect VT3-2 in the Bay of Biscay roundnose grenadier and codling did not differ significantly from each other in disturbance response and vertical positioning (Fig. 5b). However, they clearly differed in locomotion behavior (p<0.005), with roundnose grenadier showing less frequently station holding and more often drifting and forward movement than the codling.

b. Differences only in locomotion and vertical positioning (Fig. 6a)

In roundnose grenadier disturbance responses did not vary significantly between the ROV transects VT3-1 in the Bay of Biscay and ME16-1 on the Mid Atlantic Ridge (Table 1). In both cases only few individuals were recorded as being entirely undisturbed (10-21 %) and 67-87 % of all the disturbed individuals encountered responded to the vehicles at far distance. Both the locomotion behavior and the vertical positioning registered prior to disturbance responses differed significantly between the two habitats (locomotion: p=0.0005; vertical positioning: p<0.025). Roundnose grenadier occurred much higher above the bottom and showed a much higher rate of drifting on the ridge site. Station holding was frequently registered in the Bay of Biscay habitat, whereas it did not occur on the ridge site.

c. Differences in disturbance response and natural behavior between habitats and species (Fig. 6b)

The false boarfish showed significantly more disturbance responses (p<0.05), reacting more frequently at far distances during ROV transect VT1-1 in the Bay of Biscay compared to ROV transect ME4-2-1 on the Mid-Atlantic Ridge. In the latter habitat this species was positioned slightly higher above the bottom (p=0.08 for well- and far-above bottom categories combined) and showed a significant difference in locomotion behavior (p<0.005) with much less station holding and a higher rate of forward movement. Compared to the co-occurring codling in the Bay of Biscay transect, false boarfish showed significantly less disturbance (p<0.005), a much more frequent positioning well or far above the bottom (p<0.0001) and significantly more drifting and less station holding (p<0.0001)



Fig. 5. Disturbance responses, vertical positioning above bottom and locomotion behavior in (a) codling during two ROV transects on the northern Mid-Atlantic Ridge and (b) roundnose grenadier and codling during a ROV transect in Belle Isle Canyon, Bay of Biscay

Deep-Sea Fish Behavioral Responses to Underwater Vehicles: Differences Among Vehicles, Habitats and Species



Fig. 6. Disturbance responses, vertical positioning above bottom and locomotion behavior in (a) roundnose grenadier during two ROV transects in Belle Isle Canyon, Bay of Biscay (left), and on the northern Mid-Atlantic Ridge (right) and (b) false boarfish (left and middle) and codling (right) during two transects, one on Mériadzek Terrace, Bay of Biscay (VT1-1) and the other one on the Mid-Atlantic Ridge (ME4-2-1)

#### 4. Discussion and conclusions

#### Deep-sea fish disturbance responses

The underwater vehicles involved in this study elicited disturbance responses in deep-sea fishes encountered during bottom transects that can be best interpreted as avoidance or flight behavior. Clear signs of attraction to the UV's as they have been reported elsewhere (e.g., Stoner et al. 2007; Moore et al. 2008) were not observed. No longer vehicle stops and no point or selective long-term observations (e.g., by following individual fish) were conducted during the dive transects. In the studies presented here behavioral recordings were only made during

the fishes' appearance on the forward directing video screen during transects. It is well possible that additional disturbance responses occurred at larger distances before appearance or after the fish disappeared on the screen, but those were not recorded. Apart from these obvious restrictions, the registration and subsequent quantitative comparison of disturbance responses recorded during UV video transects is a solid method to investigate the influences of various factors such as different vehicles, habitats, or species on the frequency and intensity of evoked reactions (see also, Lorance et al. 2002, Uiblein et al. 2002, 2003).

While the manned submersible did not evoke any response in codling (first case study), they responded considerably disturbed when encountered in the same area with an ROV. A large portion of the disturbance responses happened at far distance or even before encounter indicating early detection, before the main illumination focus reached the fish. Sound may therefore be seen as a main source of disturbance. No exact comparative measurements are however available of the light and sound intensity produced by the two vehicles during those dives. Also, the possibilities cannot be ruled out that the signals acted in combination and that other disturbance sources such as, e.g., pressure waves produced by the moving vehicle body were involved, too. The present findings provide however no evidence that the much larger-bodied manned submersible elicited a comparatively higher disturbance response than any of the four ROV's used, whereas an opposite effect was demonstrated in the first case study.

In orange roughy, light may play an important role in addition to sound in eliciting disturbance responses, because a considerable portion of the reactions occurred at short distances only. Interestingly, the responsiveness to the ROV Bathysaurus decreased between the two adjacent habitats on the ridge. No differences in natural behavior (vertical positioning and locomotion) were observed. One additional difference, however, was a much higher density of orange roughy during the first transect, indicating aggregation formation. Does orange roughy remain particularly vigilant when residing in dense conspecific aggregations? During transects with the manned submersible Nautile an aggregation of orange roughy in the central St. Nazaire canyon did not differ in disturbance responses from conspecifics encountered in the peripheral area (Lorance et al. 2002, Uiblein et al. 2003). Aggregation formation in this species may be related to rather different activities such as resting, spawning or feeding (Lorance et al. 2002). More detailed studies of this ROV dive in the area of the northern Mid-Atlantic Ridge are planned that shall also include comparisons with roundnose grenadier and associated habitat conditions encountered during these transects.

Depth may be an important factor influencing disturbance responses, as can be concluded from the behavior of codling during ROV transects in the Bay of Biscay. These results corroborate with behavioral observations of the northern cutthroat eel *Synaphobranchus kaupii* which also showed more frequent disturbance responses at a deeper located dive in the Bay of Biscay (Uiblein et al. 2002, 2003). The latter species shows a deeper-bigger pattern, hence larger fish living at greater depth have a larger sensory surface that should facilitate signal perception. Also, as food becomes scarcer with larger depths, fish need to pay more attention to environmental stimuli. Both these argumentations may also apply to codling, however, more field and biological data would be necessary to test these assumptions.

Species differences in disturbance responses during single dive transects provide the best evidence for the importance of intrinsic, organism-dependent factors that need to be considered when studying anthropogenic disturbance. Codling showed no response during the manned submersible dive in the Bay of Biscay (OB22), while roundnose grenadier responded considerably and hence may be more sensitive to the signals emitted from this vehicle. It reacted mainly at far distance or immediately before encounter what points towards the perception of rather far-ranging signals (e.g., rather noise than light). On the other hand,

234

codling showed considerable disturbance responses when confronted with an ROV. In the same situation, false boarfish responded to a lesser extent. These three taxa differ fundamentally from each other in their biology: the codling typically holds station close to soft bottoms, the false boarfish prefers to swim or drift closely to shelter provided by hard bottom structures and corals, while the roundnose grenadier is more flexible showing different locomotion behavior and vertical positioning depending on habitat context. Among these three species/species groups, false boarfish appears least prone to predation risk, also given their rather high body (see also Moore et al. 2008). Probably the response to UV's reveals also something about a species' vigilance and assessment of predation risk.

#### Deep-sea fish disturbance responses and natural behavior: the full picture

When disturbance responses are properly identified, recorded and analyzed, natural behaviour can be studied separately thus allowing to gain insights into the ecology of deepsea fishes even in the presence of anthropogenic influences. To illustrate this, four case studies were conducted, three elaborating different aspects of natural behavior (locomotion, vertical positioning) with disturbance effects remaining constant and one with all three behaviors varying. In the first two instances only locomotion varied for codling between two separated transects during an ROV dive on the Mid-Atlantic Ridge and for roundnose grenadier and codling during a single ROV transect in the Bay of Biscay. These data indicate that while species clearly differ among each other ("species-specific" behavior), it is also of high importance to understand their behavioral flexibility in adaptation to different habitats. Behavioral flexibility or plasticity allows a choice among different locomotion modes and to select those that fit best to the prevailing conditions in the respective habitat. For instance, less station holding and increased inactivity ("sit and wait") as exemplified by codling in one of two ridge habitats (Fig. 5a) should allow efficient, energy-saving foraging when currents are weak or absent and food abundance is relatively high.

As deep-sea fishes are behaviorally flexible, one can expect to find considerable differences among contrasting habitats, as demonstrated for the roundnose grenadier by ROV dives in the Bay of Biscay and the Mid-Atlantic Ridge. While disturbance responses remained rather similar in both areas, the fish displayed more drifting and no station holding and were positioned significantly higher in the water column on the ridge. This reflects obviously behavioral adjustment to typical ridge conditions (see also, Zaferman 1992) with food particles arriving at the bottom mainly through the water column, while food input deriving from the productive shelf areas is lacking.

A rather complex picture of deep-sea fish behavioral ecology is obtained when all behaviors differ and different habitats are contrasted with different species or species groups, like in the last case study. False boarfish from habitats in the Bay of Biscay and the Mid-Atlantic Ridge were compared showing less disturbance responses, a slightly higher vertical position, less station holding, and more forward movement on the ridge site. The boarfish's behavior in the Bay of Biscay clearly contrasts with codling during the same transect, the latter showing a higher disturbance response, a position on or very close to the bottom, and more station holding. Interpretations are however complicated through one (or several) additional factor(s) that need to be considered in this as well as in the anterior case study featuring roundnose grenadier, because two different UV's were used.

#### Towards optimizing *in situ* behavioral ecology of deep-sea fishes and related research

A promising approach towards reaching best possible interpretations of what deep-sea fishes do, why they do it, and how they respond to human-induced environmental changes is to consider all influential external and internal factors in the data analysis and in the

interpretation of the results. The central method to approach this goal is to analyze videorecordings made during UV transects based on detailed description, categorization and registration of the entire behavior observed with special emphasis to separate human-induced responses from natural behavior, followed by statistical comparisons. Additional data on the biology and ecology of the target species, the physical and biological environment, and the effects and possible impacts of anthropogenic disturbance need to be integrated, too.

To reduce complexity, the number of influential variables should be minimized whenever possible. Optimally, the same design models of UV's should be used during all dives that need to be compared. Dive transects, video recordings, and data analysis should follow standardized protocols. During each transect representative size measurements combined with estimates of absolute swimming speed should be obtained from each studied fish species. Visually well identifiable species should be preferably selected for study so to minimize possible informational noise introduced by species differences within composed groups. Groups of closely related species should be used only exceptionally, when *in situ* species identification is impossible and the species have a very similar body structure, hence similar behavior can be expected. Short video or photographic close-ups of each individual fish from problematic species groups should be taken (preferably by a second camera) to visualize diagnostic details helpful for species identification. Advice and assistance from taxonomists specialized in problematic fish groups should be gathered.

Use of different UV's in comparative studies cannot be recommended, because it may turn out to be difficult, if not impossible, to adjust for disturbance effects. Most certainly more than a single signal source of disturbance needs to be considered. Experimental manipulation of light, sound, and vehicle velocity, but possibly also the magnetic field, singly or in combination, might certainly assist to better understand the relative importance of these potential sources of disturbance (see also Stoner et al. 2007). However, for full control of disturbance effects from UV's, one would also need to investigate the receiver bias and in particular the sensory equipment (Popper & Hastings 2009) and reaction norms (Tuomainen & Candolin 2010) which may differ considerably among fish species, populations, size classes, and ontogenetic stages.

The longer the encounter with an UV the more increases the chance of interactions and evocation of disturbance responses. During longer UV stops, odor plumes deriving from collected organisms or bait brought along may be formed and scavengers may be attracted (Trenkel & Lorance 2011). If point observation are made during longer stops of an UV, these data should be treated separately from transect data. Also, when stationary, the vehicle itself may be perceived in quite different ways than when transitionally encountered during transects and disturbance responses may change and in some cases shift from avoidance to attraction or even to aggression (see for instance, Moore et al. 2008). Observations of deep-sea fishes deriving from longer-term interactions with UV's are certainly interesting *per se*, but may not always contribute to properly understand natural behavior. To reduce interactions it may be of advantage to position the vehicle firmly on the ground and switch off the motors for behavioral observations close to the bottom. During point observations in the open water as well as close to the bottom switching off the illumination and use of infrared light combined with infrared-sensitive cameras should be considered (Widder et al. 2005).

As stated initially, investigations of the effects of UV's on deep-sea fish behavior have important implications for many other studies of deep-sea fishes, as for instance, *in situ* assessments of abundances, populations dynamics, habitat associations, community structure, and patterns of biological diversity (Stoner et al. 2008). Hence the suggestions and

recommendations towards optimization of *in situ* behavioral ecology may prove useful also for broader applications in deep-sea fish research and management.

#### 5. Summary

An important prerequisite for in situ ecological investigations of deep-sea fishes using underwater vehicles (UV's) is to distinguish between disturbance responses elicited by the vehicles and undisturbed natural behavior. Nine case studies deriving from ten video transects along deep bottoms of the North Atlantic (Bay of Biscay, Mid-Atlantic Ridge) with a manned submersible and three remotely operated vehicles (ROV's) are presented to demonstrate differences in behavioral disturbance between vehicles, habitats, and species. Three species, roundnose grenadier (Coryphaenoides rupestris), orange roughy (Hoplostethus atlanticus) and false boarfish (Neocyttus helgae), and codling, a group of closely related species (North Atlantic codling, Lepidion eques, being the most common), were studied. During each UV transect recordings of disturbance responses and two activity patterns shown by undisturbed fishes, vertical positioning in the water column and locomotion mode, were made. Each behavior was subdivided into several categories and analyzed quantitatively using sample sizes larger than 18 individuals per species/species group and transect. Codling showed no disturbance responses to a manned submersible, while reacting intensely to a ROV during two transects performed in the same area. When the same UV was used, clear differences in disturbance responses were found between both adjacent dive transects and species/species groups indicating habitat- and species-specific responsiveness to signals emitted by the vehicle, in particular sound and light, but possibly also other sources. In three additional case studies, disturbance responses remained rather constant between transects or species, but natural behavior differed. The final study provides the fullest picture with all three behaviors differing, the interpretations being however complicated by the fact that different vehicles were used in different habitats. The findings are discussed emphasizing the significance of in situ quantitative behavioral studies of UV-based video transects in deep-sea fish ecology and related research fields. Detailed suggestions and recommendations towards optimization of vehicle-disturbance control and observation techniques are provided.

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Autonomous Underwater Vehicles (AUVs) are remarkable machines that revolutionized the process of gathering ocean data. Their major breakthroughs resulted from successful developments of complementary technologies to overcome the challenges associated with autonomous operation in harsh environments. Most of these advances aimed at reaching new application scenarios and decreasing the cost of ocean data collection, by reducing ship time and automating the process of data gathering with accurate geo location. With the present capabilities, some novel paradigms are already being employed to further exploit the on board intelligence, by making decisions on line based on real time interpretation of sensor data. This book collects a set of self contained chapters covering different aspects of AUV technology and applications in more detail than is commonly found in journal and conference papers. They are divided into three main sections, addressing innovative vehicle design, navigation and control techniques, and mission preparation and analysis. The progress conveyed in these chapters is inspiring, providing glimpses into what might be the future for vehicle technology and applications.

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