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Mouth morphology and behavioural responses of cultured turbot towards food pellets of different sizes and moisture content

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Abstract

The importance of food pellet size and texture in pre-capture selectivity and subsequent ingestion by juvenile turbot, *Scophthalmus maximus* (Rafinesque), was investigated. Measurements of mouth parts and body lengths were taken to investigate their relation to preferred pellet sizes. Two fish size classes, with mean lengths of 123.9 ± 2.5 mm and 161.3 ± 2.9 mm were tested. Behavioural responses of individual fish offered pellets of five different diameters (2.0, 3.5, 5.0, 8.0 and 13.0 mm) and two moisture levels (10.9% and 28.3%) were investigated. Responses were classed as follows: no reaction; orientation; incomplete approach; complete approach and capture, and scored accordingly. A broad range of acceptable pellet sizes was observed in both fish size groups. In the smaller sized group, acceptable pellet diameters ranged between 4.0% and 6.5% of total fish length, or 40% of mouth gape. This decreased to 20% of mouth gape, or between 2.2% and 5.0% of total fish length in the larger fish group. Juvenile turbot demonstrated an ability to accurately select pellets within the acceptable range and pellets outside this range were largely ignored, eliciting few pre-capture behaviours.

Keywords: *Scophthalmus maximus*; Mouth morphology; Behavioural responses

1. Introduction

Morphological limitations imposed on the feeding behaviour of fish ultimately affect somatic growth (Wankowski, 1979). Feeding behaviour of individual fish species is an

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important consideration in feed development, and the understanding gained from behavioural studies, particularly of salmonids, has been used to improve performance under culture conditions (Wankowski, 1981; Stradmeyer et al., 1988; Smith et al., 1995). Diets formulated specifically for marine flatfish have become available as the culture of these species has increased. Research attention has focussed mainly on nutritional requirements (Andersen and Alsted, 1991; Nijhof, 1991; Devesa, 1994) with some reference to the role of behavioural selectivity (Martinez Cordero et al., 1994).

While diet composition is clearly important in determining both consumption and assimilation, physical attractiveness of food to the fish is also important in maximising consumption. Behavioural responses have been employed in the determination of prey preferences with varying physical characteristics (Knights, 1983; Stradmeyer, 1989; Linnér and Brännäs, 1994) and a number of different techniques have been employed. Although these techniques are most easily used with species that hold their position in the water column during feeding, it is essential that pellets be optimised for each species individually as many show divergent feeding behaviours (Brännäs and Alanärä, 1992).

An understanding of the behavioural processes involved in the ingestion of a food item is essential in enabling the production of pellets appropriate to optimising selection and minimising wastage. This involves an understanding of the individual behavioural components of each stage, from the identification of a potential prey item through to the ingestion or rejection of this for consumption, as restrictions in pellet design on feeding can be imposed at any of these stages (Wankowski and Thorpe, 1979). Furthermore, the optimal delivery of pellets to fish may also be influenced by behavioural considerations (Smith et al., 1995). Previous workers have shown that turbot, *Scophthalmus maximus*, select their prey on the basis of size, shape, colour and movement. Gustatory characteristics such as texture, taste and smell are also important for some other fish species (Stradmeyer, 1992). Experiments have been carried out on some of the factors governing prey selection by wild turbot, brought into the laboratory for observation (De Groot, 1971; Holmes and Gibson, 1986). Knutsen (1992) investigated the feeding response of turbot larvae using behavioural responses. This is the first study of the behavioural responses of juvenile hatchery reared turbot to pelleted diets. The primary aim is to assess the ability of two different size classes of turbot to identify, capture and ingest food pellets of different sizes and textures by examining the behaviour directed towards each pellet type. A secondary aim is to relate feed selectivity to the mechanics of the feeding apparatus and associated morphometric limitations.

2. Materials and methods

2.1. Experimental design

Sixteen hatchery-reared juvenile turbot, *S. maximus*, eight each of two size categories, were used in this study. One week prior to the start of the trial, morphological measurement of mouthparts were taken to the nearest 0.1 mm using a vernier callipers. Head width (mm), mouth gape (maximal internal dimension between the inner lateral edges of the maxillaries, mm), upper and lower maxillary length (mm) and internal mouth height (mm) were measured for each fish individually. Weights were measured to the nearest 0.1 g on an electronic balance, and lengths (from the tip of the closed mandible to the posterior end of the caudal fin) were measured to the nearest 1.0 mm on a measuring board. Measurements were also taken from 25 additional fish, randomly selected from the source population, to provide a size range for comparative purposes. The experimental fish were then transferred from their communal holding tank to individual, 50-l white PVC, observation tanks. Seawater was continuously recirculated through these tanks and the temperature, which was not controlled, ranged from 14.8° to 15.9 °C, and the photoperiod regime was 12 h light:12 h dark.

Fish of similar weight and length were selected for each experimental size category. The smaller sized category had a mean weight ($\pm$ s.d.) of 34.7 $\pm$ 0.7 g, and a mean length of 123.9 $\pm$ 2.5 mm. The larger sized category had a mean weight ($\pm$ s.d.) of 80.1 $\pm$ 1.6 g and a mean length of 161.3 $\pm$ 2.9 mm. Fish were acclimatised to the experimental conditions for one week, during which time they were fed approximately 1% of their body weight per day, comprised of a mixture of dry and moist pellets of various sizes. Behavioural observations of feeding responses were made over four further days. All fish were starved for 24 h prior to testing. At observational feeding times, pellets were introduced to the tank, through a 200-mm tube, within the field of view of the fish (Fujimoto et al., 1992) at a distance of approximately 150 mm and a height of approximately 300 mm. Eight different pellets were tested during each session in random order of size and composition. The behavioural responses of individual fish to pellets of different sizes and moisture levels were recorded using a video recorder mounted over the tank. Fish were then starved for a further 24 h before the next behavioural observation time.

2.2. Experimental feeds

The low moisture diet, termed ‘dry’, tested in this trial was a commercially available marine flatfish diet with a moisture content of 10.9%, and the high moisture diet, termed ‘moist’ was formulated to contain 28.3% moisture. The composition of the dry portion of the two diets was the same (51.5% protein, 16.8% oil, 1.1% fibre and 9.6% ash). Additional moisture was introduced to the fish meal in the form of sterile distilled water and 1% dilution of triptone Soya agar was used as the binding agent. This mixture was extruded through dies of various diameters (2.0, 3.5, 5.0, 8.0 and 13.0 mm), and coated in 3% (by weight) fish oil, identical to that used in the formulation of the dry diet. These diameters correspond to a range from 1.6% to 10.5%, and 1.2% to 8.1% of fish length for the small and large size classes of fish, respectively (Table 1).

2.3. Analysis of behavioural investigations

Video recordings of the feeding of each fish at each feeding time were made and these were viewed subsequent to the conclusion of this study. The response of each fish to each pellet type was scored using a behavioural scoring system first used by Neill and Cullen (1974) and subsequently modified for use with turbot by Holmes and Gibson (1986).

Table 1
The range of pellet diameters (mm) tested, and their relative proportions of mean fish length and mean mouth gape of each size category

Pellet diameter (mm)	2.0	3.5	5.0	8.0	13.0
% of fish length					
small size group	1.6	2.8	4.0	6.5	10.5
large size group	1.2	2.2	3.1	5.0	8.1
% of mouth gape					
small size group	10.1	17.7	25.3	40.4	65.7
large size group	7.6	13.3	19.0	30.4	49.4

Scores awarded to each behaviour type were 0: no response to the pellet, 1: orientation towards the pellet, 2: incomplete approach of the pellet, 3: complete approach of the pellet, 4: capture of the pellet. The number of pellets rejected subsequent to capture was also recorded.

2.4. Data analysis

Linear regression analysis was used to investigate the relationships between various morphological measurements. Friedman's test for related samples was used to compare the behavioural responses of fish to pellets of different sizes. Multiple comparisons were carried out using the Mann–Whitney test and the bonferroni procedure was used to correct for multiple tests using a significance of 0.05. All analyses were carried out using SPSS for Windows (Release 7.0).

3. Results

3.1. Morphology

Mouth gape was significantly associated with both upper and lower mandibles (Fig. 1). The relationship between mouth gape (B) (mm) and the upper mandible length (U_m) (mm) was found to be highly significant ($R^2 = 0.79$, $df = 37$, $P < 0.001$) and was described by the equation:

$$B = 0.73U_m - 0.06$$

The association between mouth gape and lower mandible length (U_l) (mm) was also highly significant ($R^2 = 0.80$, $df = 37$, $P < 0.001$) and was described by the equation:

$$B = 0.68U_l + 0.45$$

turbot mouth gape was also found to be significantly associated with total fish length ($R^2 = 0.77$, $df = 37$, $P < 0.001$) (Fig. 2). The relationship between mouth gape (B) (mm) and

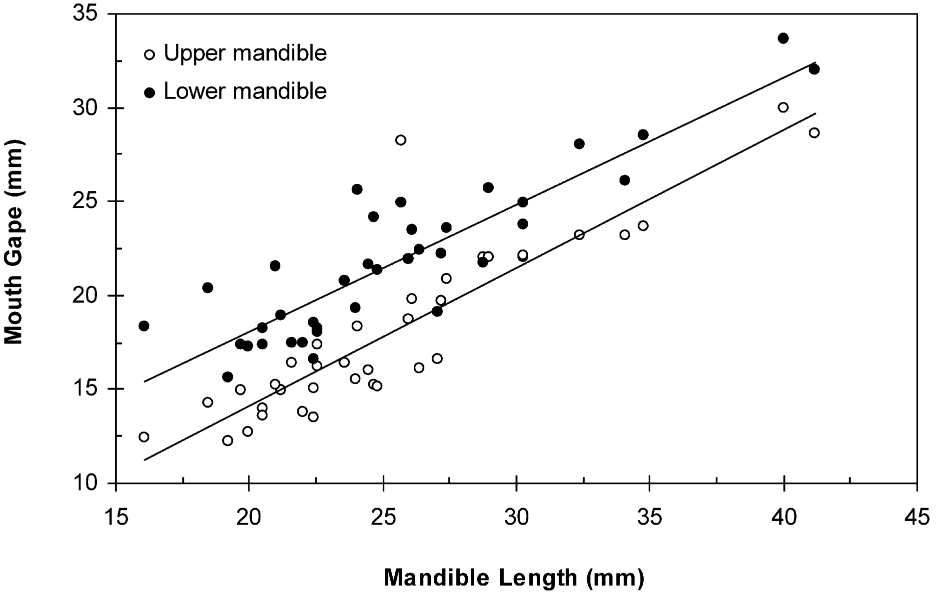

Fig. 1. The relationship between mouth gape and both upper and lower mandible lengths in juvenile turbot ($n = 41$).

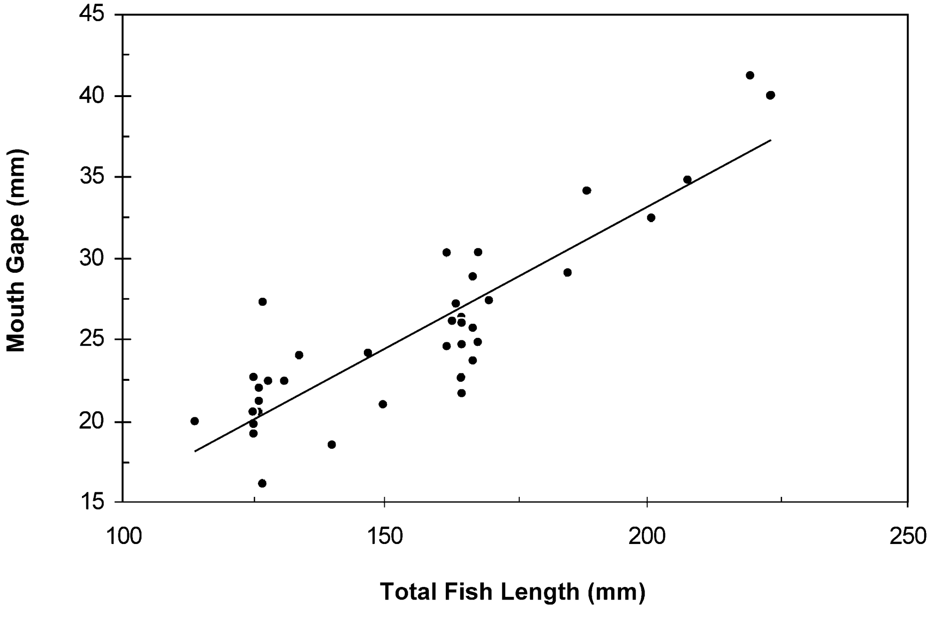

Fig. 2. The relationship between turbot mouth gape and total fish length in juvenile turbot ($n = 41$).

total fish length (TFL) (mm) for juvenile turbot (mean weight 74.6 ± 47.6 g) was described by the equation:

$$B = 0.17\ \mathrm{TFL} - 0.17$$

3.2. Behavioural responses

3.2.1. Small size group

There was no significant difference in the recorded response scores to different dry pellets smaller than 8.0 mm in diameter ($H = 1.82$, $df = 3$, $P > 0.05$) (Fig. 3). There was however, a significant difference between the scores recorded for this pellet size range and those pellets 13.0 mm in diameter ($U = 150.00$, $df = 71$, $P < 0.05$) which showed significantly lower scored behaviours. The pellet sizes for which the highest scored behaviours were recorded were those in the mid-size range with diameters of 5.0 and 8.0 mm. The frequency distribution of behaviours demonstrated that successful capture of pellets was similar for all pellet sizes, with the exception of those pellets with 13.0 mm diameters where observed behavioural responses were reduced (Fig. 4(A)). Rejection of pellets subsequent to capture was observed only with the 5.0 and 8.0 mm pellets, and of these 22.2% and 28.6%, respectively, were rejected. These were, however, the dry pellet sizes favoured by turbot of this size, and their lengths correspond to 4.0% to 6.5% of total fish length or 25.3% to 40.4% of mouth gape. The percentage of these pellets offered to the small size turbot group that were successfully ingested were 64.3% and 63.6%, respectively, as compared to successful

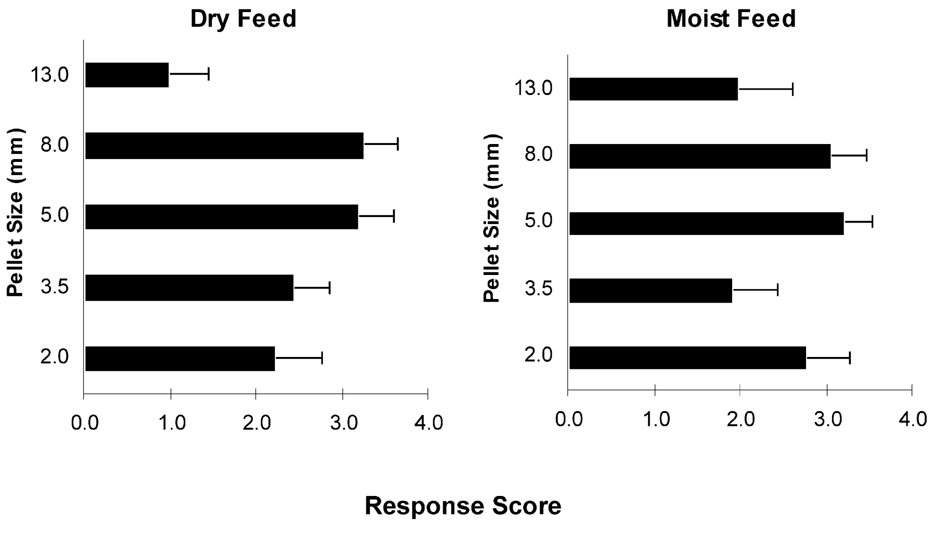

Fig. 3. Mean response scores of turbot in the small size group to dry and moist pellets of different sizes. Scores were 0: no response, 1: orientation towards the pellet, 2: incomplete approach of the pellet, 3: complete approach of the pellet, and 4: capture of the pellet. (Horizontal bars represent one s.e.).

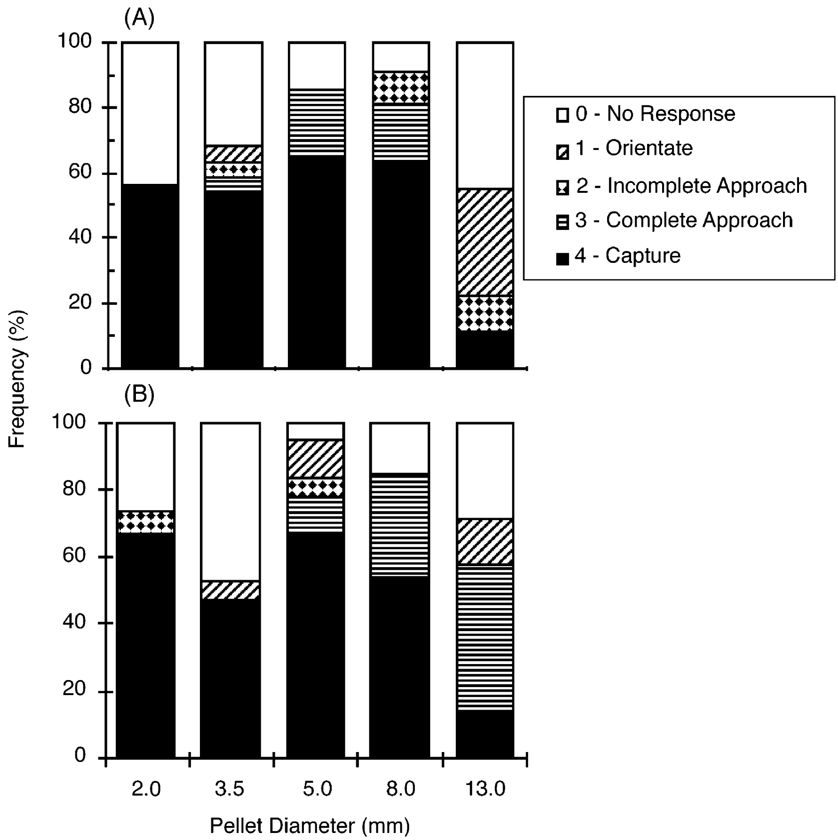

Fig. 4. Frequency distribution of behavioural response types towards dry pellets (A) and moist pellets (B) of different sizes by fish in the small size class.

ingestion of 56.3% of 2.0 mm and 54.6% of 3.5 mm pellets offered to the turbot, and just 11.1% of 13.0 mm pellets.

The range of pellet sizes acceptable to the small fish increased with the 'moist' diet (Fig. 3) and there was no significant difference in the response scores awarded to any moist pellet sizes ($H = 6.44$, $df = 4$, $P > 0.05$). Of the moist pellets successfully captured, those in the 3.5, 5.0 and 8.0 mm size classes were the only ones where subsequent rejection was recorded, at rates of 12.5%, 16.7% and 28.6%, respectively. Rejection of the other two pellet sizes subsequent to capture was 0.0%. The moist pellet sizes examined obtaining the highest mean score were those with diameters of 5.0 and 8.0 mm. These corresponded to 4.0% and 6.5% of total fish length and 25.3% and 40.4% of mouth gape, and 66.7% and 53.9%, respectively, of these pellets offered were successfully ingested.

3.2.2. Large size group

There was a significant difference in the scores awarded to the behavioural responses by the larger turbot towards dry feed pellets of different diameters ($H = 17.74$, $df = 4$,

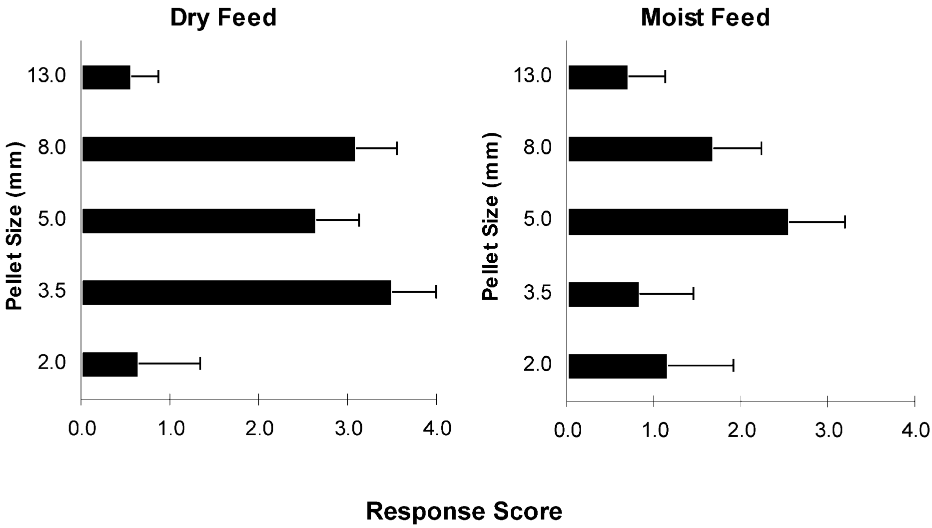

Fig. 5. Mean response scores of turbot in the large size group to dry and moist pellets of different sizes. Scores were 0: no response, 1: orientation towards the pellet, 2: incomplete approach of the pellet, 3: complete approach of the pellet, and 4: capture of the pellet. (Horizontal bars represent one s.e.).

$P<0.001$) (Fig. 5). The highest average response scores were recorded for pellets with diameters between 3.5 and 5.0 mm. There was no significant difference in the response scores awarded to behaviours towards pellets with diameters of 3.5, 5.0 or 8.0 mm ($H=1.36$, $df=2$, $P>0.05$). These data were therefore pooled and on this basis the response scores awarded to these sizes were significantly higher than those awarded to pellets of 2.0 mm ($U=28$, $df=36$, $P<0.01$) and 13.0 mm ($U=37.50$, $df=37$, $P<0.001$).

The frequency distributions of the different behavioural responses towards dry pellets of different sizes are shown in Fig. 6(A). A greater number of successful captures were recorded for pellets between 3.5 and 8.0 mm than larger or smaller pellets. This corresponded to pellet sizes between 2.2% and 5.0% of total fish length, or 13.3% and 30.4% of mouth gape. Of the dry pellets successfully attacked in this trial by the larger sized fish, 22.2% of the 5.0 mm and 28.6% of the 8.0 mm pellets were subsequently rejected. This rejection behaviour was not observed with any other pellet size. 83.3% of 3.5 mm dry pellets offered to these turbot were successfully ingested, and despite the recorded rejection rates, 46.7% and 50.0% of 5.0 and 8.0 mm pellets, respectively, were successfully ingested.

There was no significant difference in the response scores awarded to moist pellets of any of the different sizes ($H=3.13$, $df=4$, $P>0.05$) (Fig. 5) when they were presented to the large turbot. In comparison to the situation observed with dry pellets, however, a higher proportion of complete attacks were directed towards just one size of moist pellets—the 5.0 mm pellet size (Fig. 6(B)). This corresponded to 3.1% of total fish length or 19.0% of mouth gape. Of the moist pellets captured, 40.0% of 5.0 mm and 33.3% of 8.0 mm were subsequently rejected, a higher proportion than rejected of the dry pellets,

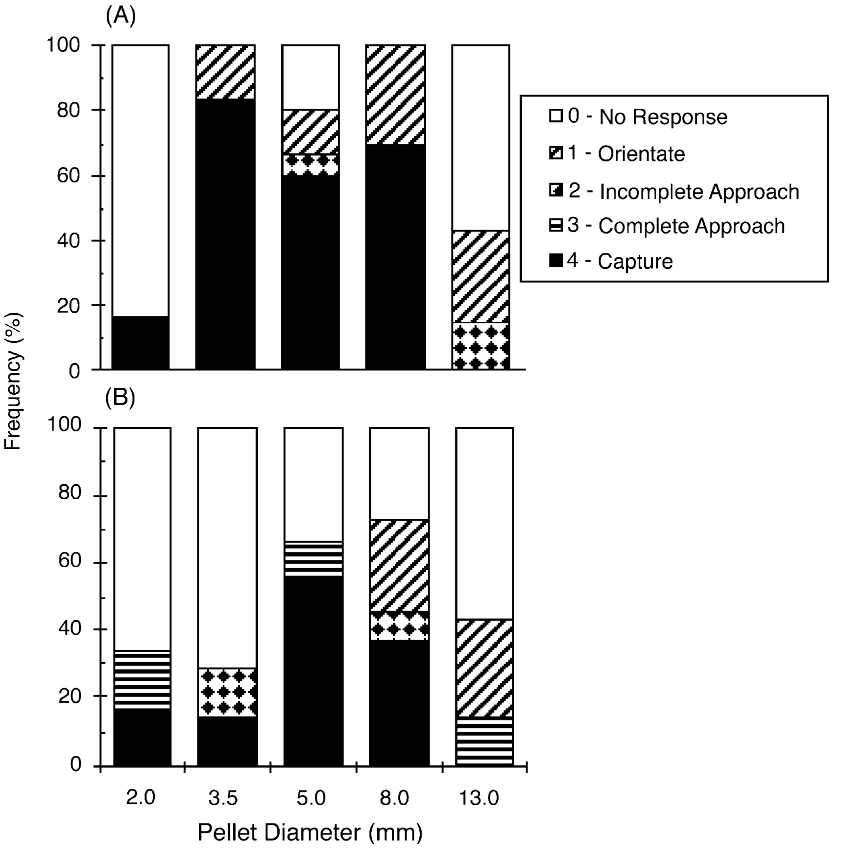

Fig. 6. The frequency distribution of behavioural response types towards dry pellets (A) and moist pellets (B) of different sizes by fish in the large size class.

although the percentage of successful ingestion was still higher for these pellets than for pellets of the remaining three sizes, 55.6% and 30.0%, respectively.

4. Discussion

A significant linear association between mouth gape and total body length in juvenile turbot, *S. maximus* (Rafinesque), was revealed by investigation of mouth morphology and total body lengths between 11.4 and 22.4 cm. Relationships between these two parameters are not uncommon (Wankowski, 1979), although the two are not always related linearly (Erzini et al., 1997). Mouth gape, measured here as the maximum horizontal internal distance between the mandibles, was strongly dependent on the individual lengths of both upper and lower mandibles (Fig. 1). Mouth gape of each individual turbot was also strongly associated with the total length of each fish.

At the beginning of exogenous feeding there is an olfactory component to the feed selectivity behaviour of turbot (Knutsen, 1992). Following metamorphosis, they become visual feeders, and wild turbot actively pursue free swimming prey in the water column (Yazdani, 1969) during daylight hours. Prey animals include crustaceans and other fish species (De Groot, 1971) and their jaws are adapted for the capture of these mobile prey items. The preferred prey size of wild turbot increases with increasing fish length, which is correlated with the expansion of the buccal-pharyngeal cavity during juvenile growth (Braber and de Groot, 1973). The relative lengths of turbot buccal cavity and body length change as fish grow from 100 through 300 mm, and selected natural prey items change in length, above which size their relative size remains constant.

A broad range of acceptable pellet sizes for juvenile turbot was observed in this study. The pellet sizes that elicited the highest scoring behaviours by turbot in this study were equivalent to 40% of mouth gape, or between 4.0% and 6.5% of body length for the small sized turbot. In the larger turbot group, the pellets seen to elicit the highest scores corresponded to 20% of mouth gape, or 2.0–5.0% of body length. This is somewhat higher than the preferred pellet size of salmonid species where the reported optimum size is 2% of total fork length (Wankowski and Thorpe, 1979; Brännäs and Alanärä, 1992), and eels where the reported optimum is as low as 0.4% (Knights, 1983). These differences reflect the differences in the mechanics of capture and ingestion of prey by these species. During prey capture in turbot, the jaws extend forward creating a siphon through which the prey item is sucked into the buccal cavity (Holmes and Gibson, 1983). It is the dramatic increase in volume resulting from this extension that permits turbot, and other flatfish, to consume comparatively large prey items.

Ingestion of a food item by a fish is the conclusion of a chain of behavioural events initiated by the identification of a prey item. The main behavioural components of the turbot feeding response have been identified as orientation, approach and capture (Holmes and Gibson, 1983). Reared and wild turbot show differences in motivation to attack and in efficiencies of prey recognition and capture (Ellis et al., 1997). Poor feeding success is seen in inexperienced cultured individuals, but motivation and efficiency improve with experience. Prey movement and appendage movement are important characteristics in prey identification and initiation of the behavioural feeding response by wild turbot. Farmed turbot, by contrast, prefer pellets and even stones shaped like pellets to natural shrimp and are most likely to select prey items of which they have had previous experience (Ellis et al., 1997). In this present study, juvenile turbot demonstrated an ability to identify pellets within the acceptable range from the total range of pellets offered to them. Pellets outside this range were largely ignored, and the majority of attempts at prey capture resulted in successful ingestion. Zero (no response) and 4 (successful capture) were the most common behaviours observed (Figs. 4 and 6). Such selectivity, which helps to minimise foraging effort, is not a characteristic of all cultured fish species. Both length and diameter of pellets are important in the identification and ingestion of prey by Atlantic salmon *S. salar*, however, smaller pellets are more easily ingested than larger ones, even though the latter are visually more attractive (Stradmeyer et al., 1988; Smith et al., 1995). Feed range selectivity may, however, be governed by hunger levels, for example, in the case of sticklebacks, *Spinachia spinachia*, where, when feeding motivation declines feed size selectivity increases (Croy and Hughes, 1991).

The wide range of acceptable pellet sizes seen in this study is advantageous in the manufacture of pellets due to the wide variation in growth of cultured turbot and resulting large size frequency distributions within tanks (Irwin et al., 1999). In this respect, a narrow acceptable size range would necessitate the feeding of wide pellet ranges to turbot groups, ultimately increasing wastage, or the regular grading of groups to maintain small size frequency distributions.

References

Andersen, N.G., Alsted, N.S., 1991. Growth and body composition of turbot (*Scophthalmus maximus* (L.)) in relation to different lipid / protein ratios in the diet. In: Kaushik, S.J., Luquet, P. (Eds.), Fish Nutrition in Practise. INRA, Paris, pp. 89–96.

Braber, L., de Groot, S.J., 1973. On the morphology of the alimentary tract of flatfishes (*Pleuronecteformes*). J. Fish Biol. 5, 147–153.

Brännäs, E., Alanärä, A., 1992. Feeding behaviour of the Arctic charr in comparison with the rainbow trout. Aquaculture 105, 53–59.

Croy, M.I., Hughes, R.N., 1991. The influence of hunger on feeding behaviour and on the acquisition of learned foraging skills by the fifteen-spined stickleback, *Spinachia spinachia* L. Anim. Behav. 41, 161–170.

De Groot, S.J., 1971. On the inter-relationships between morphology of the alimentary tract, food and feeding behaviour in flatfishes. Neth. J. Sea Res. 5, 121–196.

Devesa, S., 1994. Nutrition and feeding of cultured turbot (*Scophthalmus maximus* L.). In: Lavens, P., Remmerswaal, R.A.M. (Eds.), Turbot Culture: Problems and Prospects. European Aquaculture Society, Belgium, pp. 81–92.

Ellis, T., Howell, B.R., Hughes, R.N., 1997. Comparative feeding efficiency of wild and hatchery-reared turbot, *Scophthalmus maximus*. The first International Symposium on Stock Enhancement and Sea Ranching, Bergen, Norway, 8–11 September, p. 140.

Erzini, K., Goncalves, J.M.S., Bentes, L., Lino, P.G., 1997. Fish mouth dimensions and size selectivity in a Portuguese longline fishery. J. Appl. Ichthyol. 13, 41–44.

Fujimoto, M., Satoh, Y., Morishita, F., 1992. Measurement of background-visual field in flatfishes by CRT display method. Mar. Behav. Physiol. 19, 285–290.

Holmes, R.A., Gibson, R.N., 1983. A comparison of predatory behaviour of flatfish. Anim. Behav. 31, 1244–1255.

Holmes, R.A., Gibson, R.N., 1986. Visual cues determining prey selection by the turbot, *Scophthalmus maximus* L. J. Fish Biol. 29, 49–58.

Irwin, S., O'Halloran, J., FitzGerald, R.D., 1999. Stocking density, growth and growth variation in juvenile turbot, *Scophthalmus maximus* (Rafinesque). Aquaculture 178, 77–88.

Knights, B., 1983. Food particle-size preferences and feeding behaviour in warmwater aquaculture of European eel, *Anguila anguila*. Aquaculture 30, 173–190.

Knutsen, J.A., 1992. Feeding behaviour of North sea turbot (*Scophthalmus maximus*) and Dover sole (*Solea solea*) larvae elicited by chemical stimuli. Mar. Biol. 113, 543–548.

Linnér, J., Brännäs, E., 1994. Behavioural response to commercial food of different sizes and self-initiated food size selection by Arctic charr. Trans. Am. Fish. Soc. 123, 416–422.

Martinez Cordero, F.J., Beveridge, M.C.M., Muir, J.F., 1994. A note on the behaviour of adult Atlantic halibut, *Hippoglossus hippoglossus* (L.) in sea cages. Aquacult. Fish. Manage. 25, 475–481.

Neill, S.R., Cullen, J.M., 1974. Experiments on whether schooling by their prey affects the hunting behaviour of cephalopod and fish predators. J. Zool. London 172, 549–569.

Nijhof, M., 1991. Effects of body weight on diet requirements of turbot (*Scophthalmus maximus*) with special references to feed intake and body composition. In: Kaushik, S.J., Luquet, P. (Eds.), Fish Nutrition in Practise. INRA, Paris, pp. 89–96.

Smith, I.P., Metcalfe, N.B., Huntingford, F.A., 1995. The effects of food pellet dimensions on feeding responses by Atlantic salmon (*Salmo salar* L.) in a marine net pen. Aquaculture 130, 167–175.

Stradmeyer, L., 1989. A behavioural method to test feeding responses of fish to pelleted diets. Aquaculture 79, 303–310.

Stradmeyer, L., 1992. Appearance and taste of pellets influence feeding behaviour of Atlantic salmon. In: Thorpe, J.E., Huntingford, F.A. (Eds.), The Importance of Feeding Behaviour for the Efficient Culture of Salmonid Fishes. World Aquaculture Society, Baton Rouge, pp. 21–28.

Stradmeyer, L., Metcalfe, N.B., Thorpe, J.E., 1988. Effect of food pellet shape and texture on the feeding response of juvenile Atlantic salmon. Aquaculture 73, 217–228.

Wankowski, J.W.J., 1979. Morphological limitations and prey size selectivity, and growth response of juvenile Atlantic salmon, *Salmo salar*. J. Fish. Biol. 14, 89–100.

Wankowski, J.W.J., 1981. Behavioural aspects of predation by juvenile Atlantic salmon (*Salmo salar* L.) on particulate, drifting prey. Anim. Behav. 29, 557–571.

Wankowski, J.W.J., Thorpe, J.E., 1979. The role of food particle size in the growth of juvenile Atlantic salmon (*Salmo salar* L.). J. Fish Biol. 14, 351–370.

Yazdani, G.M., 1969. Adaptation of the jaws of flatfish (*Pleuronectiformes*). J. Zool. London 159, 181–222.