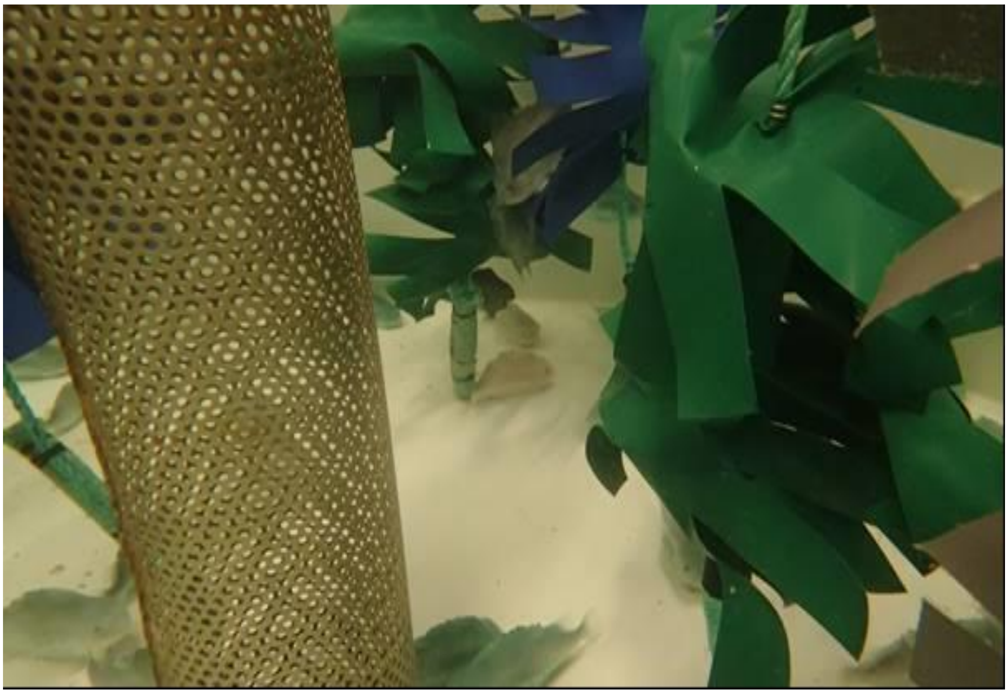
Gildeskål Forskningsstasjon a.s

Pilot study:
Do Lumpfish (*Cyclopterus lumpus* L.) exhibit a behavioural preference to attach to different coloured substrates manufactured with either polypropylene (PP) or polyvinyl chloride (PVC) as they grow?



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April 2019

Version 1.0

Preface

Specific objectives of this study were to assess behavioural and physical attachment preference of lumpfish offered different coloured substrates manufactured with two different materials. The study will also elucidate the surface area of substrates required by lumpfish as they grow.

To this end, the main objectives of this present study were:

1. Assess attachment preferences of lumpfish offered PVC and PP substrates of different colours.
2. Assess growth and performance of lumpfish and record biometric measurements of lumpfish as they grow.
3. Calculate the surface area of each substrate used as the fish grow.

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TITLE	Substrates	PROJECT LEADER	
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Abstract/Summary

To investigate the possible difference in behavioural and physical attachment preference of lumpfish offered different coloured substrates manufactured with two different materials, 40 lumpfish with a mean (±SD) weight of 142.3 ± 4.7 g were stocked in a 3.5 m^3 circular flow-through tank. To the tank, substrates were placed to offer attachment. Two substrates were green 450g PVC, two were blue PP and two were grey PP (manufactured by XPO Arctic AS).
Growth of the lumpfish showed positive trends throughout the study period with the mean weights of the experimental group increasing at each sampling time point and no mortalities were recorded throughout the study.
Results from the present study show that lumpfish showed no preference to the type of substrate offered for attachment. In addition, the results indicated that the colour of substrate did not influence attachment preference.
In addition, there appeared to be no difference in the degree of biofouling between PVC and PP when exposed to marine coastal areas.

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1.0 Introduction

The biological control of sea lice using cleaner-fish has recently become a feasible option due to the increased occurrence of resistance towards medical treatments in salmon lice, *Lepeophtheirus salmonis* (Igboeli *et al.*, 2012; Torrissen *et al*., 2013), the reduced public acceptance of chemotherapeutic use in food production and the urgent need for an effective and sustainable method of parasite control in Atlantic salmon aquaculture (Denholm *et al.*, 2002; Treasurer, 2002). As a cold-water cleaner-fish alternative the common lumpfish (Cyclopterus lumpus L.) is currently used to control sea lice infestations. The challenge in using lumpfish as biological delousing agents is to optimize their grazing potential and previous studies have shown that lumpfish do graze on attached sea lice from Atlantic salmon (Imsland et al., 2014abc, 2015a). However, efficient use of lumpfish in terms of the proportion of fish that graze sea lice, is dependent upon the establishment and maintenance of healthy and robust populations. Previous studies have shown that lumpfish require some type of substrate to attach to (Imsland *et al*, 2015b). The physical form of the lumpfish is highly characteristic. The head is thick and short, with a blunted nose, and a high dorsal crest that covers the first dorsal fin entirely. The body is covered in scale-less skin, with rows of dark tubercles along the body (Davenport 1985). The pelvic fins constitute a ventral suction disc, allowing fish to rest on rocky substrate, kelp and vegetation. The lumpfish is notable among fishes for hatching with a functional mouth and a well-developed digestive system (Timeyko 1986). Juveniles are typically found among kelp during the first year of their life, both attached and free floating (Ingólfsson and Kristjánsson, 2002). About 1 year after hatching, they are found in pelagic habitats, but frequently found in floating seaweed (Ingólfsson and Kristjánsson, 2002; Vandendriessche et al. 2007) using their suction disc to adhere to the seaweed surface when resting. In addition, a previous study has shown that wild juvenile lumpfish forage using one of two modes: they can actively search for prey while swimming or they can ‘sit-and-wait’ for prey while clinging to the substrate using a ventral adhesive disk (Killen et al., 2007). The study suggested that juvenile lumpfish forage in a manner that reduces activity and conserves space in their limited aerobic scope. The authors noted that this behavioural flexibility is of great benefit to this species, as it allows young individuals to divert energy towards growth as opposed to activity.

Mimicking their natural requirements for surface adhesion, lumpfish stocked in sea pens with Atlantic salmon will require some form of substrate to attach to when resting. Substrates may also provide shelter for lumpfish during periods of inactivity and/or extreme environmental perturbations. If lumpfish are subjected to perturbations that are likely to result in increased stress response, then there is a higher probability that stressed lumpfish can become prone to disease particularly bacterial agents. Commercial producers are setting out lumpfish in commercial salmon farms at around 20g as demand is high for this species. If small lumpfish are to be used commercially then it is important that the environmental conditions are considered prior to transfer to maintain healthy populations.

There are several commercially available artificial substrates recently developed, used in commercial salmon cages presently. However, very little research has been done to optimize their use for the target species for which they have been designed for. In addition, most are manufactured with PVC. The study aimed to compare PVC substrates with substrates manufactured with PP which is free of chloride and potentially easier to recycle.

Specific objectives of this study were to assess behavioural and physical attachment preference of lumpfish offered different coloured substrates manufactured with two different materials. The study will also elucidate the surface area of substrates required by lumpfish as they grow.

To this end, the main objectives of this present study were:

1. Assess attachment preferences of lumpfish offered PVC and PP substrates of different colours.
2. Assess growth and performance of lumpfish and record biometric measurements of lumpfish as they grow.
3. Calculate the surface area of each substrate used as the fish grow.

2.0 Methods

2.1 Lumpfish and feeding.

Lumpfish with a mean (±SD) weight of 142.3 ± 4.7 g were used in the study. The fish originated from fertilised eggs from Senja Akvakultursenter AS, Tromsø.
One-week prior to the start of the trial, 40 fish were individually weighed and distributed into a 3.5 m^3 circular flow-through tank labelled M3.
At regular intervals throughout the study period (with no starvation period) all lumpfish from the tank were individually weighed on a precise laboratory scale (0.5 g) (Ohaus Valor 2000, Switzerland) to determine the average wet body weight (WBW) of lumpfish. Feeding ration was calculated considering the number of fish in the tank (minus any mortalities) and the average WBW of lumpfish. The amount (g) of food to be fed to the fish at a given feeding ration was calculated as:

$$Fa = Fb \times Fr/100,$$

where *Fa* is the daily food amount (g) which should be supplied to the tank, *Fb* is the fish biomass (g), and *Fr* is the weekly daily feeding rate (2% of the biomass in each tank).

2.2 Study design

2.2.1 Biometric measurements and growth performance

All lumpfish from all groups were individually weighed and fork-length recorded routinely during the trial period. In addition, during weighing, the height of each fish was measured (mm) using a digital calliper (Cocraft Vernier, USA) and the width and length measured by placing the fish on a flat surface and adjusting two rulers to record width and length (Image 1). Individual surface area was calculated for each fish based on fish height and width.
Data derived from these measurements was used to construct a table for optimal deployment of lumpfish in commercial salmon cages taking into consideration the amount of surface attachment area required as the fish grow.
The tank was checked for mortalities every day. Any mortalities present were removed, and their weight and length recorded.

Image 1 Measuring length and width of lumpfish

Condition factor (K) of individual lumpfish (calculated at each weighing interval) was defined as:

$$K = 100 * W = L3$$

where W is the weight (g) of the fish and L the corresponding total length (cm).

2.2.2 Substrates

Two different materials were used to manufacture the substrates used in the study. Two substrates were made of green coloured 450g PVC and four others made with two different colours (blue and grey) polypropylene (XPO Arctic AS). The substrates where randomly deployed beside each other in the centre of the tank for them to be exposed equally to the water current in each tank (Image 2).

Image 2 The three different types of substrates deployed during the study.

Prior to deployment, the total surface area of each substrate was calculated by measuring every available surface area in cm2 and each was labelled with an identification number. During regular intervals throughout the study period, the number of lumpfish attached to each substrate were counted by netting the fish and substrate together and transferring them to a holding tank. In addition to the number of fish attached at each sampling time point, the total area used of each substrate and the percentage of fish attached was calculated. All fish were counted, and their weight, length, width and height recorded as described in the previous section after which the fish were returned to the tank and the substrates re-deployed.

2.2.3 Water current

The water current of the tank was set to a predetermined value using a Geopacks Advanced Stream Flow Meter (Geopacks, Devon, UK.) and adjusting the water intake valve to reach the desired current speed of 5.0 cm s^{-1}.

2.2.4 Study conditions

Sea water was pumped from 70 m depth at a temperature of between 5.4 and 8.5 °C and oxygen saturation was maintained above 80%. The study period was from January to February 2018

2.2.5 Water quality

Water temperature and oxygen concentration was recorded daily using a Handy Polaris 2 probe (OxyGuard International A/S.

2.3 Biofouling rate of PP and PVC material

Sections (1 m^2) of PVC and PP material were mounted on wooden frames and positioned at a depth of 3 meters at Gifas small-scale facility Langholmen for a period of 45 days. At regular intervals during the deployment period, the frames were carefully removed, and a digital high-resolution image taken of each after which the frames were re-deployed. At the end of the study period, the images were qualitatively compared to evaluate potential differences in the biofouling rate between them.

3.0 Results and discussion

3.1 Growth and performance

Mean weight recorded throughout the study period can be seen in figure 1. Growth of the lumpfish showed positive trends throughout the study period with the mean weights of the experimental group increasing at each sampling time point and no mortalities were recorded throughout the study. Previous studies have shown that growth rates of small lumpfish are generally higher compared to fish of a larger size class (Imsland *et al* 2014a) and the growth rates observed in this study were similar to growth rates from previous studies (Imsland *et al.* 2015*ab*).

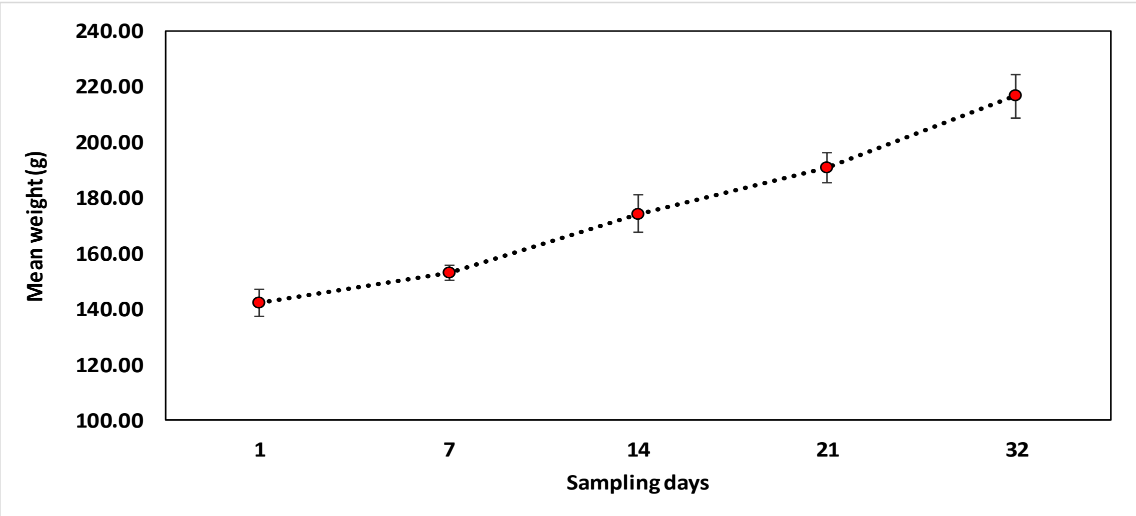

Figure 1 Mean weight (g) of lumpfish maintained at 5 cm s^{-1}. Values represent means ± S.D (N = 40).

There was moderate variation in K throughout the study period (figure 2). This compares with previous studies where variations were attributed to food choice selection between individuals within populations (Imsland *et al*, 2014a and 2015a). There exists a high degree of variability in K within lumpfish populations. The value of K is influenced by several factors such as age of fish, sex, season, stage of maturation, fullness of gut, type of food consumed, amount of fat reserve and degree of muscular development. The K values recorded suggests that the fish spent similar amount of energy whilst attached to both thin and thick material substrates of different colours. Previous studies have shown that as lumpfish grew, they showed no preference to thicker material substrates at higher water speeds suggesting that they can maintain position even at relatively higher water speeds (Imsland & Reynolds *et al,* 2018). In addition, there were no mortalities for all three groups during the study.

3.2 Attachment preference

There were no significant differences between the percentage of fish attached to each of the three types of substrates as they grew (figure 3). The percentage of fish attached to the green PVC substrate ranged between 14.6% at day 7 to 32.0% at day 21 whilst the percentage of fish attached to the XPO blue substrate ranged between 12.7% at day 1 to 33.8% at day 21. The percentage of fish attached to the grey XPO material ranged between 15.9% and 38.5%.

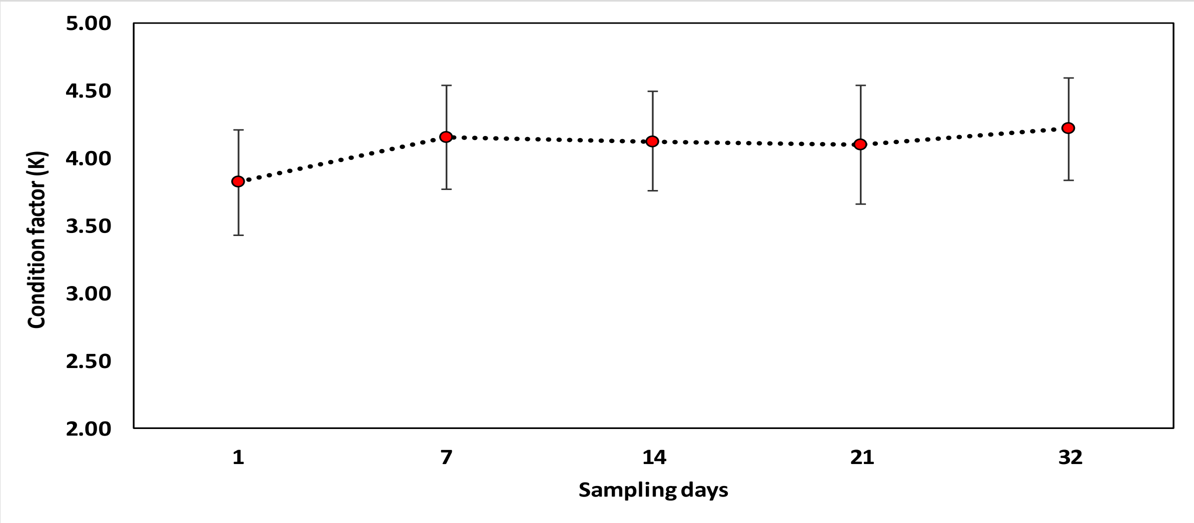

Figure 2 Mean Condition factor (K) of lumpfish maintained at 5 cm s^{-1}. Values represent means ± S.D (N = 40).

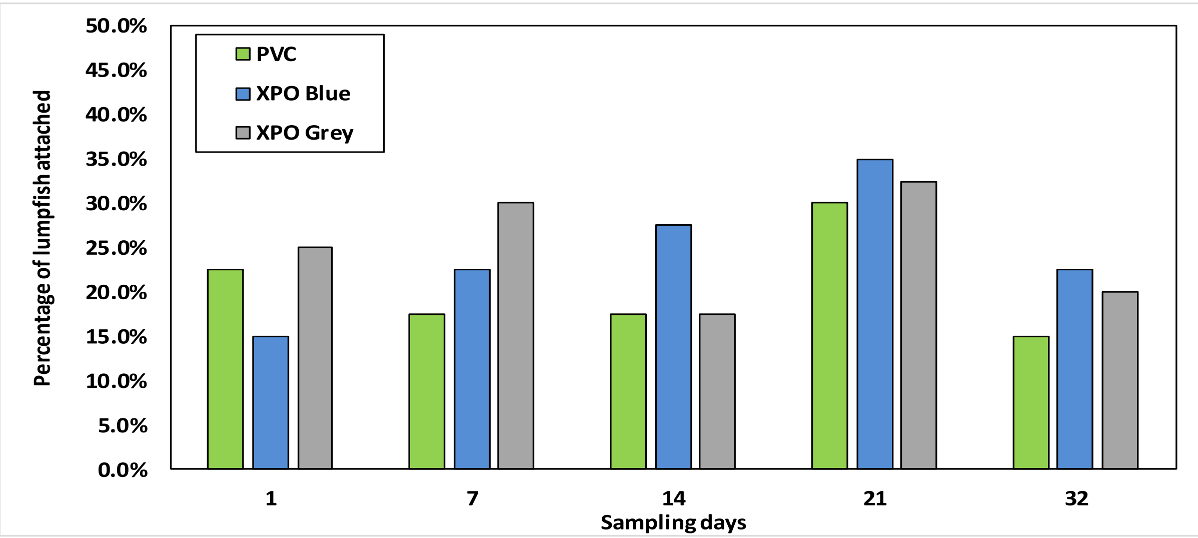

Figure 3 Percentage of lumpfish attached to each substrate at each sampling day.

The percentage of available substrate surface area used by lumpfish at each sampling time point can be seen in figure 4. There was an increase in surface area of each type of substrate used in all three treatments as the fish grew up to day 21. There was a reduction in surface area used at day 32 which correlates to less fish found attached (see figure 3).

Results from then study show that there were no differences in attachment preference for lumpfish and attachment choice was irrespective of weight gain and colour of substrate (image 3). Attachment by lumpfish is initiated by the contraction of muscles running between the sucker skeleton and the hydroid arch. A previous study by Davenport and Thorsteinsson

(1990) showed that a vertical force of 98.3 – 101.6 KN m^2 was required to dislodge suckers of mature lumpfish (> 1 kg) and that on polypropylene surfaces, a force of 5 N was required before sliding was observed. These forces will be influenced by the type of material and its degree of smoothness the fish are attached to (Davenport and Thorsteinsson, 1990). The authors go on to state that the forces observed are strong enough to hold a fish in place when exposed to high currents, particularly given the fact that fish generally face head on into currents thus minimising the body surface area exposed to the drag reducing effects of water flow (Davenport and Thorsteinsson, 1990). A previous study undertaken at Gifas (Imsland & Reynolds *et al*, 2018) was in line with these observations even though the study observed substrate choice by much smaller and younger fish. It may well be that the forces required to dislodge large mature lumpfish are similar for smaller fish or the water currents assessed in the study were too low for the fish to be challenged to remain attached.

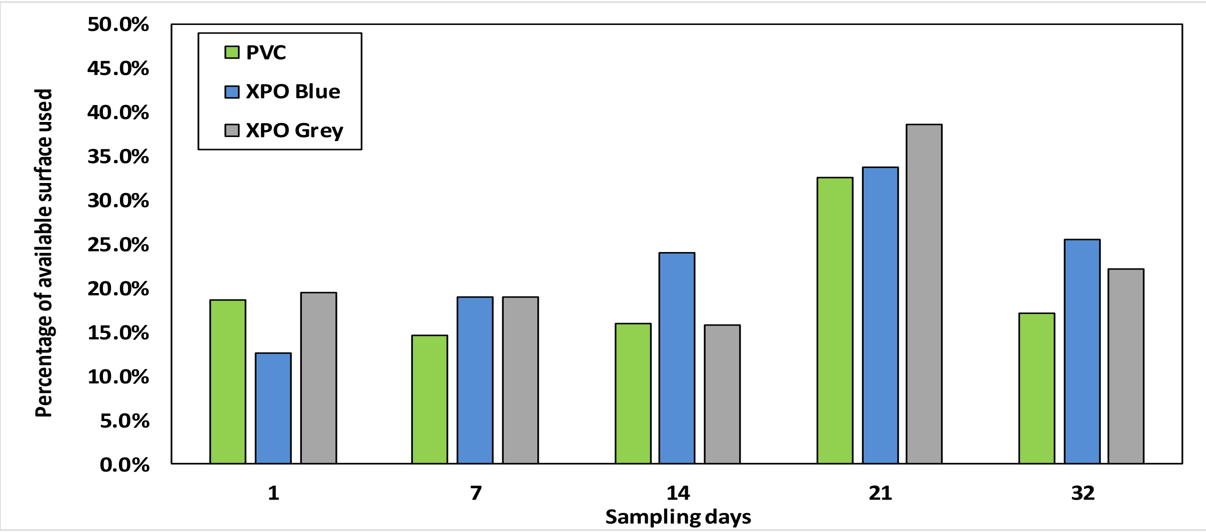

Figure 4 Percentage of lumpfish attached to each substrate at each sampling day.

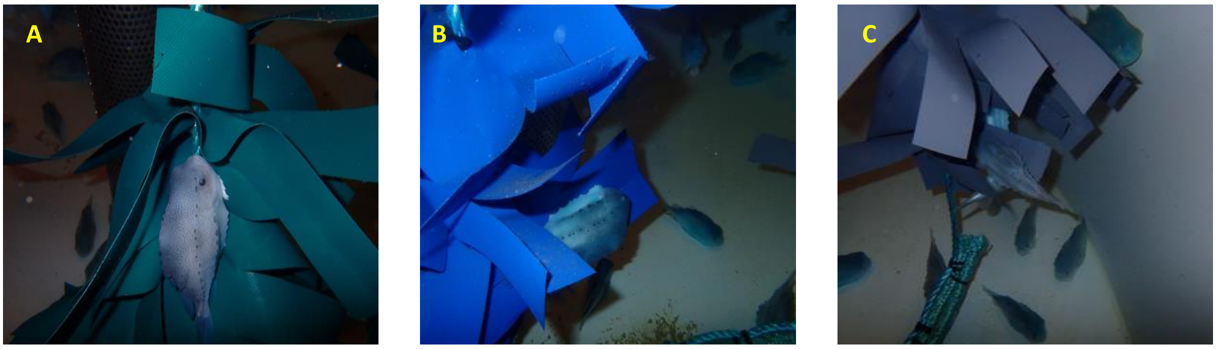

Image 3 The three different substrates used during the study. (A) green PVC; (B) blue PP (XPO) and (C) grey PP (XPO).

There was little information on the thickness of the XPO materials assessed during this study and material weight/thickness may have to be considered if substrates are to be used in high energy sites with average water speeds in excess of 20 cm s^{-1}. To date, there have been no studies assessing substrate choice by lumpfish at high water speeds and more research is required to fully elucidate potential choices.

It is also worth noting that small lumpfish may be directly challenged when transferred to an open net-pen environment in winter time with low winter water temperatures when most commercial salmon farms are routinely setting out juvenile lumpfish at an average weight of 20g in commercial cages. Juvenile lumpfish spend their early stages in the physically challenging intertidal zone where they are reported to grow rapidly before migrating to colder feeding grounds (Hedeholm *et al.*, 2014) where they may better exploit the differences in temperature. A previous study has shown that wild juvenile lumpfish forage using one of two modes: they can actively search for prey while swimming or they can 'sit-and-wait' for prey while clinging to the substrate using a ventral adhesive disk (Killen et al., 2007). The study suggested that juvenile lumpfish forage in a manner that reduces activity and conserves space in their limited aerobic scope. The authors noted that this behavioural flexibility is of great benefit to this species. This behavioural response observed by wild juveniles has implications for lumpfish stocked in commercial salmon cages. It is known that substrates must be provided for the fish to attach and rest when desired. This natural behaviour may help to maintain healthy and robust populations and thus may even enhance their lice grazing potential.

In the wild, juvenile lumpfish are typically found among kelp, both attached and free-floating during the first year of their life (Ingólfsson 2000; Ingólfsson and Kristjánsson 2002), but are also found attached to flat and rigid substrates like blue mussel Mytilus edulis and stones (Moring 1989). In general, members of the family Cyclopteridae use their ventral adhesive disc to adhere to rocks, vegetation and other available substrates (Moring 1989). Ingólfsson (2000) investigated the colonization of lumpfish in coastal floating seaweed clumps, originating from the intertidal zone. A negative correlation between the density of lumpfish and clump weight was found, and the authors speculated whether this might indicate some form of territorialism in lumpfish (i.e. fry excluding other individuals from their immediate vicinity) or cannibalism, common in floating seaweed or kelp. Based on the data, Ingólfsson (2000) deduced that floating seaweed and kelp are an important habitat for the species during the first year, while older fry, although pelagic, are not found associated with floating seaweed. It is important when deploying substrates for lumpfish in commercial salmon cages that factors such as territorialism are considered by providing sufficient surface area for the fish with an additional calculate surface area to dilute or eliminate potential territorial effects.

It is important that substrates used in commercial salmon cages are deployed in a position that facilities close contact between both lumpfish and salmon to facilitate lice grazing. Currently salmon farmers are using artificial substrates which are generally suspended in the water column (image 4). These "curtain kelps" allow the lumpfish to attach and are placed in the cage to facilitate close contact by both species.

Image 4 Example of artificial curtain kelp used in commercial salmon cages (photo, NorseAqua. http://norseakva.com/).

3.3 Biofouling of PVC and PP (XPO) materials

There were no significant differences in the speed and degree of colonization by marine organisms over time for PVC and XPO material of different colours (image 5). There were similar areas of biofouling at each sampling days with almost complete coverage by d 43.

The degree of colonisation was assessed qualitatively, and more detailed research would need to be undertaken to assess potential colonisation differences and /or preferences between different organisms over time.

4.0 Conclusions

Results from the present study show that lumpfish showed no preference to the type of substrate offered for attachment. In addition, the results indicated that the colour of substrate did not influence attachment preference. In addition, there appeared to be no difference in the degree of biofouling between PVC and PP when exposed to marine coastal areas.

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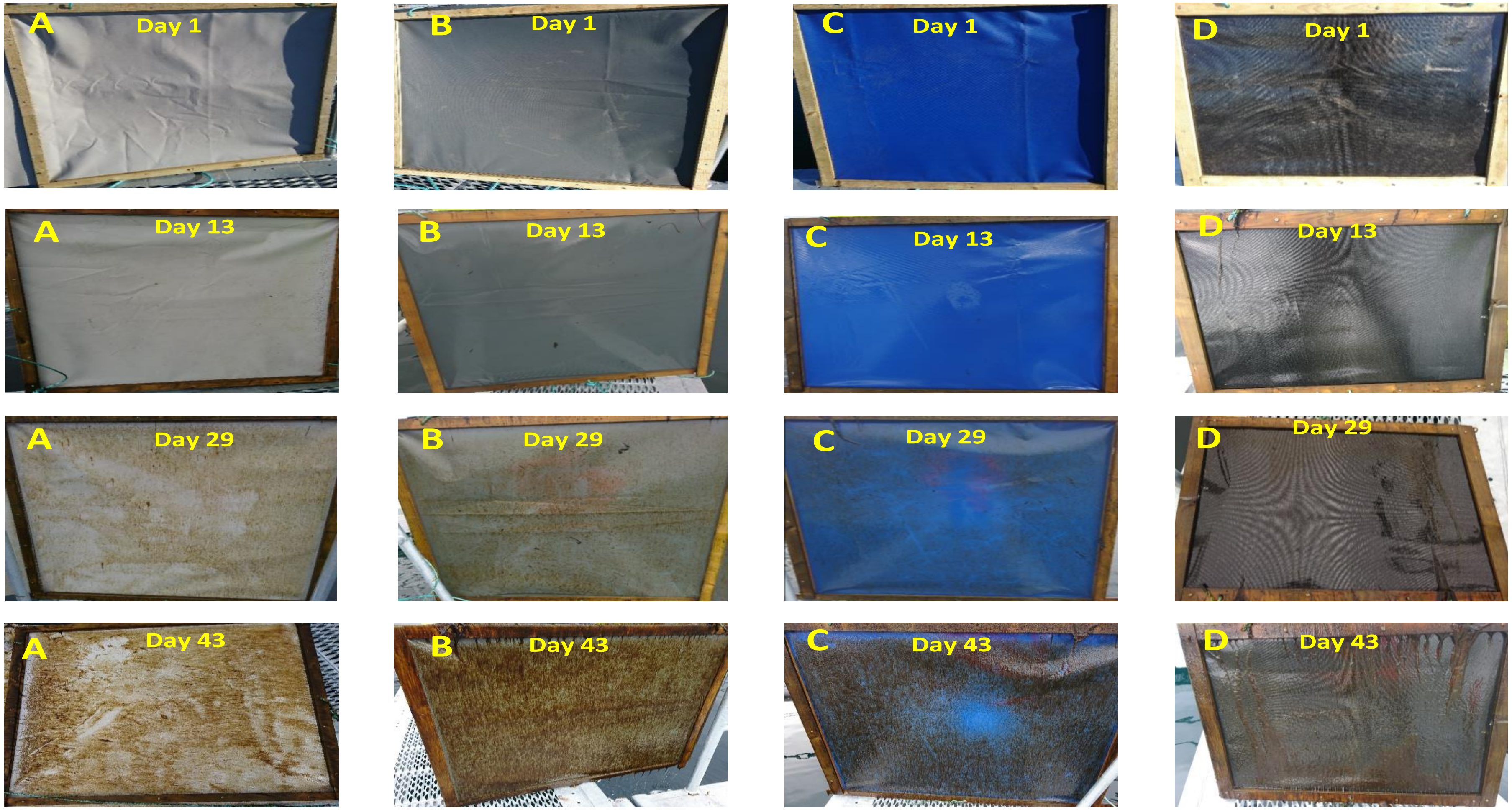

Image 5 Evaluation of colonisation by marine organisms over time for 4 substrate sections. (A) grey PVC; (B) grey PP (XPO); (C) blue PP (XPO); (D) black PP (XPO) at days 1, 13, 29 and 43 post-deployment.

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