Copepod production in a saltwater pond system: A reliable method for achievement of natural prey in start-feeding of marine fish larvae

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ABSTRACT

A high-latitude seawater pond system was restarted after 10 years absence of controlled biological production. Water supply systems for emptying and refilling the 25,000 m³ pond were installed along with a wheel filter plankton collection unit which enabled fractionation and concentration of live zooplankton, consisting mainly of various stages of copepods. A raft was centrally located in the pond, serving as a platform for hydrographical and biological sampling, water mixing, and delivery of inorganic nutrients to boost primary production. No copepod resting eggs seemed to have survived the 10-year resting period, and copepod eggs and nauplii were reintroduced with the refilling of seawater to the pond. Abundances of copepod nauplii increased about 5 months after refilling, with subsequent generations of copepodids and adult copepods. The plankton was dominated by the calanoid copepods *Acartia longiremis* and *Centropagus hamatus*. The pond was managed according to a distinct year cycle, with the natural production season limited from March to October followed by cooling, quiescence, and complete draining before refilling in February and July to prevent establishment of other planktonic organisms than copepods. About 4.6 and 45.4 billion copepod resting eggs were estimated to be ready to hatch from the pond sediment at refilling in February the second and third year of operation, respectively. Thus, the operational procedures enabled synchronous hatching of copepod nauplii during spring season for use in large start-feeding experiments with marine fish larvae. Further, 2.7 and 1.6 billion copepods of various stages were harvested during 40 and 66 days periods in 2012 and 2013, respectively. The pond has proven itself a reliable supplier of copepods which sustained complete feed delivery through the whole larval period in large-scale start-feeding trials with marine fish larvae.

1. Introduction

Copepods are the most important feed for planktivorous fish, including the larval stages of many fish species (Last, 1978, 1980; Möllmann et al., 2004). Use of copepods as feed in production of marine fish has reduced frequencies of skeleton deformities and improved pigmentation, survival, and growth rates during larval and early juvenile stages (Øiestad et al., 1985; Næss et al., 1995; Shields et al., 1999; Payne and Rippingale, 2000; Støttrup, 2000; Finn et al., 2002; Imsland et al., 2006; Koedijk et al., 2010; Liu and Xu, 2009; Busch et al., 2010; Barroso et al., 2013). Compared to commonly used live feeds like rotifers (*Brachionus* sp.) and *Artemia*, copepods are superior with respect to essential nutrients (Watanabe et al., 1983; Witt et al., 1984; Evjemo et al.,

2003; van der Meeren et al., 2008; Hamre et al., 2013). For this reason, there has recently been an increasing interest in applying copepods in larval fish rearing. This interest also includes the need to avoid nutritionally induced effects in bio-assay studies with fish larvae or other organisms that need live planktonic prey as feed (Drillet et al., 2011a), e.g. in toxicity testing of compounds of anthropogenic origin, physiological studies, or ecological assessments like impacts of ocean acidification or global warming. Copepods are key organisms in the aquatic food webs, and with regard to their use in experimental studies, attention to such prey organisms is therefore expected to increase.

Obtaining enough copepods of desired stages at a specific time has been one of the barriers for extensive use in aquaculture and experimental work with fish larvae or other copepod-feeding organisms. Therefore, establishment of reliable production methods for copepods that can meet the quantitative requirements of larval fishes is essential. Harvesting copepods from the sea is not an option as variability in abundance makes it difficult to obtain a

stable supply. In addition, wild copepods may carry parasites that can impose a threat to larval fishes (Drillet et al., 2011a). Copepod cultivation is therefore preferred, and includes both intensive production under controlled conditions and extensive rearing in tanks or ponds (Støttrup, 2003). Intensive production however, still struggles with obtaining enough copepods due to the long generation time and relatively low copepod densities in the cultures compared to rotifers and *Artemia*. However, methods for inducing quiescence in subitaneous copepod eggs and subsequent cold-storage have been developed (Drillet et al., 2006; Holmstrup et al., 2006; Højgaard et al., 2008). This allows synchronized hatching of copepod eggs in intensive cultures, which may increase the availability of copepod nauplii. Nevertheless, the nauplii still have to be reared through the naupliar and copepodid stages if to be used as a food source for larger or older fish larvae.

On the other hand, mass production of copepods in large volumes of water like mesocosms, enclosures, or ponds, has proven to provide sufficient amounts of copepods from initiation of exogenous feeding until completion of metamorphosis in larval fish (van der Meeren and Naas, 1997). Such systems have been managed as small-scale ecosystems in which the fish larvae either have been reared directly in the pond water along with the naturally occurring plankton organisms (Rognerud, 1887; Kvenseth and Øiestad, 1984; Pedersen et al., 1989; Blom et al., 1991; Engell-Sørensen et al., 2004) or more interestingly, the pond can be manipulated to enhance copepod production where the desired copepod stages can be collected and concentrated by filters for the use as feed for larval fishes (Naas et al., 1991; van der Meeren et al., 1994; Berg, 1997; Sørensen et al., 2007; Su et al., 2006).

One such pond system, the "Svartatjern" pond in Austevoll, western Norway, was in continuous operation from 1984 to 2001. During this period, a method aiming at minimizing copepod predators, depleting parasite infestation, and maximizing overwintering of copepod resting eggs in the sediments was developed. This protocol secured hatching of predictable quantities of copepod nauplii in the spring and subsequent copepodid generations for use in larval fish rearing experiments. However, since 2001 this pond had been accumulating rainwater, leaving a layer of anoxic seawater at the bottom. The present study describes the restart of this pond system with subsequent establishment of a marine copepod community and a seasonal management protocol. The restart may be equivalent to starting up a marine copepod pond from scratch, and is therefore valid for any other new establishment of similar enclosure systems to be used in aquaculture or in research.

2. Materials and methods

2.1. The "Svartatjern" pond facility

The pond "Svartatjern", located at N60°5′47″, E5° 15′05″ in the vicinity of the Institute of Marine Research – Austevoll Research Station (IMR), was originally a small freshwater lake before conversion into a marine pond that could be drained and refilled by pump systems. Svartatjern as a seawater rearing facility was established in 1984 and used for production of juvenile cod by the extensive method that same year (Naas et al., 1991; van der Meeren and Naas, 1997). From 1985, the pond was used as a copepod production unit designed for harvest of various stages of copepods to be used in ecological, genetical, and nutritional studies of larval fish (Næss et al., 1995; van der Meeren and Jørstad, 2001; van der Meeren and Moksness, 2003; van der Meeren et al., 2008). The Svartatjern facility was terminated in 2001, and all technical equipment used for pond operation was removed. Due to renewed interest in marine copepods, in 2010 the decision was made to refit the pond

for copepod production again and full operation re-established in 2011.

After reinstallation, Svartatjern had a depth of 4.5 m, an area of 10,600 m², an approximate volume of 25,000 m³, was bowl-shaped with a surface to volume ratio of 0.48, and had a layer of organic sediment at the bottom that was several metre thick in the centre of the pond. Seawards, the pond was closed by a 1 m high concrete dam with standpipes for water level control and drainage of freshwater from rain and land runoff. This drainage took place at the pond surface, preventing the saltwater to unintentionally leave the pond. To gain and preserve heat in the pond water, a thin freshwater layer of 10–20 cm was allowed to build up from rain runoff during the production season. This layer of fresh or slightly brackish water creates a strong pycnocline that will act as the glass in a greenhouse, leading to accumulation of heat from incoming sun radiation by insulating the pond water. This will result in shorter copepod generation time relative to what is expected from seasonal atmospheric and seawater temperatures.

2.2. Water supply, mixing, and drainage systems

A pump station for seawater supply, located offshore in the nearby bay (Fig. 1a), consisted of a wooden cabin on top of a pump basin bolted to two large concrete blocks resting on the sandy sea bed in the shallow bay. The water supply pipeline was a submerged PEH pipe (Ø = 355 mm) extending 270 m beyond the bay, to a depth of 35 m into the fjord. This provided seawater in the salinity range 31–34 psu and temperatures between 8 and 12 °C. A submersible Flygt channel impeller pump (type CP3127MT433, 4.7 kW, 230 V, 1445 rpm) in cast iron with up to 2 m³/min capacity (Xylem Water Solutions AS, Oslo, Norway: www.flygt.com) was installed in the pump station. To prevent potential negative biological effects in the enclosed pond water, no zinc or other metal anodes were used on the pump (Jelmert and van Leeuwen, 2000). The pump was stored on deck inside the cabin and lowered by double guide bars when connection to another pipeline used for pumping the water to the pond was needed. This was a 170 m long PEH pipe (Ø = 200 mm) coupled to two UNIK-900 wheel filters (Unik Filtersystem AS, Os, Norway: www.unikwater.com), enabling filtration down to 80 μm of all seawater pumped to the pond (Fig. 1b). This filtration excluded small planktivorous fish and other potential copepod predators. Outlets of the UNIK filters trapped considerable volumes of air and distributed fine air bubbles into the effluents. Therefore, water passing the wheel filters was collected in a 1.5 m³ circular fibreglass tank for stripping of trapped air before entering the pond through a 95 m long PEH pipe (Ø = 305 mm) ending near the bottom at the centre of Svartatjern. Removal of this air was necessary to prevent flotation of the pipe extending into Svartatjern, and possibly also aeration leading to mixing of the seawater and the surface freshwater layer in the pond.

Another submersible Flygt channel impeller pump (type CT3085MT434, 1.4 kW, 230 V, 1405 rpm with a variable-frequency drive) was placed on a raft located in the centre of Svartatjern (Fig. 1c). This pump was either used for draining the pond completely or supplying the plankton filters with pond water during filtration of copepods. It had a capacity of 1.5 m³/min and was also without zinc or other metal anodes. To prevent overload, the raft pump could not be operated at more than 45 Hz (90% of full speed). The pump was attached to a 2.5 m high metal arc on the raft deck by a stainless steel wire, pulley, and a hand-operated winch, and could be lowered to any depth position in the pond. Usually, 0.3 and 2 m depths were used for pond draining and copepod filtration, respectively. Pond water was pumped through a 95 m long PEH pipe (Ø = 200 mm), either to the UNIK-900 filters where it returned to Svartatjern through the air-stripping tank and pipe system, or directly to another 160 m long PEH pipe (Ø = 305 mm) for transfer
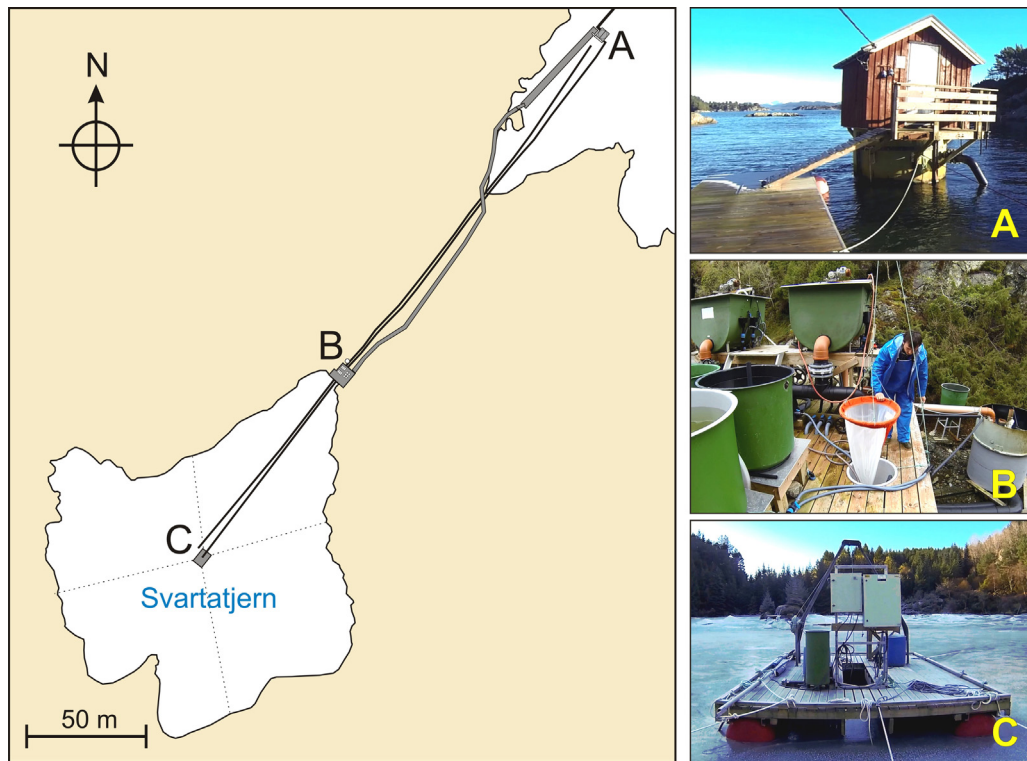
Fig. 1. Overview of the Svartatjern pond with operational facilities. The pump basin (A) is connected to an inlet pipe from the fjord, and contains a pump that is directly coupled to a supply pipe for the plankton filter system (B) so that filtered seawater can enter the pond through a pipe ending nearby a raft platform positioned in the centre of the pond (C). Another submersible pump is located at the raft platform, used for supplying the filter system during plankton collection, or for emptying the pond through an additional pipe ending in the sea outside the pump basin. To access the pond, the facility is equipped with a floating dock connected to a gangway (shown in dark grey colour).

to the sea when the pond was emptied. Flow directions were controlled by hand-operated valves placed on the filter system platform (Fig. 1b).

To prevent stratification of the seawater in the pond a stainless steel Flygt compact mixer with a jet ring (type 4620, 0.75 kW, 230 V, controlled by a variable-frequency drive) was fixed in a horizontal position at 2.5 m depth by a 5 m long vertical stainless steel pipe ($\varnothing$ = 63 mm) hinged on the raft deck. This arrangement allowed adjusting the angle of propulsion by tilting the steel pipe to the desired position.
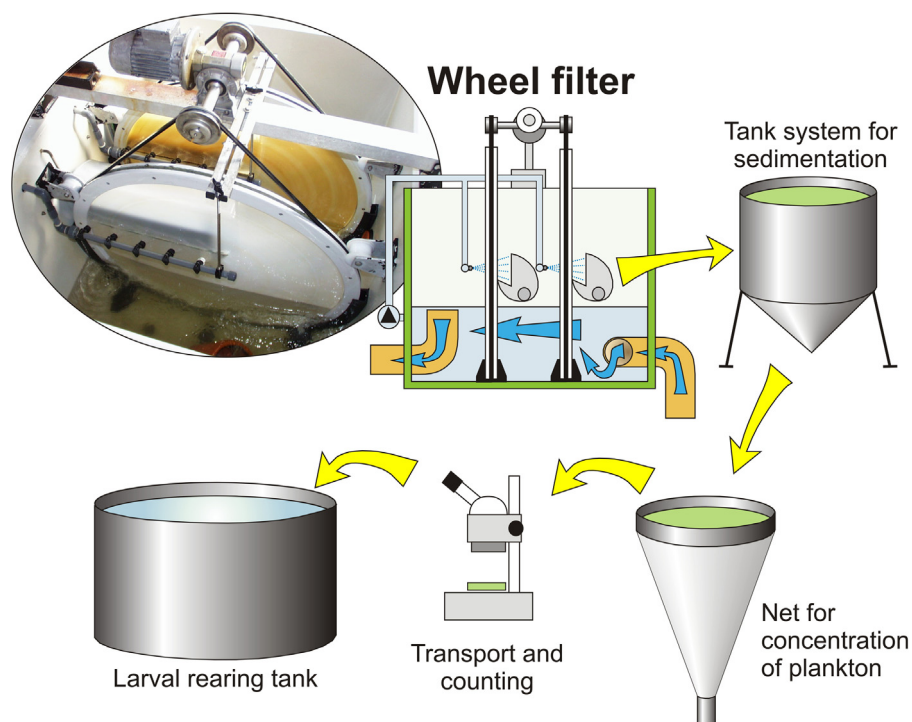
2.3. The plankton filter system

The two UNIK-900 wheel filters were placed on a wooden frame about 1.1 m above the platform deck on top of the dam at the entrance of Svartatjern (Fig. 1b). Each filter was equipped with two exchangeable filter wheels (Fig. 2) made of a plankton net mounted on a fibre glass ring ($\varnothing$ = 900 mm), separating the u-formed filter tank into three compartments. Mesh sizes from 80 to 350 μm were used in filtration of copepods. The wheel filter system could be operated both manually or automatically by a timer.

When the filters were engaged, incoming unfiltered seawater reached the first compartment where coarse-filtration through the first wheel took place as the water entered the second compartment. Next, fine-filtration occurred as the water passed through the next filter wheel of 80 μm mesh size and entered the third chamber. Here an effluent pipe of PVC plastic (90° bend, $\varnothing$ = 200 mm) determined the water level inside the filters. The wheels were continuously rotating, driven by belts from an electric engine mounted on an aluminium frame on the top of the filters (Fig. 2). Copepods and other planktonic particles were trapped on the mesh screen and brought out of water on the rotating wheels, before being flushed

off the screen into a collection box of stainless steel with a plastic lip scraping the plankton net. Flushing was carried out by spraying 80-μm filtered seawater from the third compartment through 6 nozzles mounted on a PVC pipe ($\varnothing$ = 25 mm) that covered the radius of the back of the wheel vis-à-vis the collection box. A stainless steel Grundfos CR pump (Grundfos Holding AS, Bjerringbro, Denmark: www.grundfos.com) was installed to power the flushing. From the collection boxes, the filtrate consisting of copepods and particulate matter were drained by gravity, either to waste during refilling of the pond or collected in a set of six 250-L fibreglass tanks with conical bottoms for accumulation of live copepods and sedimentation of non-living material (Fig. 2). The two size fractions of the filtrate could be directed through PEH pipes ($\varnothing$ = 32 mm) and PVC valves to the inlet in the bottom of any of the collection tanks, which would take about 50 min each to fill.

Once the collection tanks were filled, the copepod filtrates coming from the filters were automatically switched to an overflow tube back to the pond through the air-stripping tank and pipe system. A PVC standpipe ($\varnothing$ = 40 mm) was fitted to the bottom, inside the cone in each collection tank. This pipe had four holes ($\varnothing$ = 10 mm) in a ring about 15 cm above the bottom, which allowed particles settling on the walls and in the cone to stay in the collection tank when it was emptied. Sedimentation of non-living material started automatically when the tanks were filled and the flow stopped, and was necessary to achieve a clean concentrate of copepods. Due to high densities of copepods with subsequent decline in oxygen concentration and possible cannibalism (Boersma et al., 2014), it was essential that sedimentation was carried out for a short period, and therefore draining of the collection tanks started within half an hour after being filled. The design permitted the copepod filtrates to leave the collection tanks through the same tubes as they entered the tanks, and for further

Fig. 2. The principles of the plankton filtration system (slightly modified from Mangor-Jensen and Holm, 2004). From the two plankton net wheels of the filter, the collected copepods were flushed via collection boxes to sedimentation tanks, where another concentration of the copepods through a plankton net took place before transport to the larval fish rearing facility. Arrows inside the schematic drawing indicate direction of water movement, arrows outside this drawing denotes movement of collected plankton. See text for a detailed description of the system and its operation.

volume reduction the filtrates were sieved through a plankton net of 80 μm mesh size (Fig. 2) that was submerged in another tank below the platform deck (Fig. 1b). In this way, up to 1.4 m³ of filtrate could be concentrated to 10–15 L which easily was transported in an aerated container to the larval rearing facility. Copepod survival during this final concentration and transport has previously been regarded as 100% (van der Meeren et al., 2008).

2.4. The seasonal management protocol

Svartatjern is situated at high latitude, with large seasonal variations in light and temperature: ranging from 0 to 24 °C in water, −12 to 30 °C in air, and 6–18 h sunlight/day. The protocol used for managing the biological production in the pond included 1–2 complete drainages per year to prevent or reduce establishment of nuisance organisms in the system such as bivalves, gastropods, barnacles, polychaets, cnidarians, or small planktivorous fishes. Such organisms may either feed on various stages of copepods or have planktonic stages that interfere with the sizes of copepods collected in the filters for the larval fish. About 10–12 days was needed to drain the pond, and it was left empty for another week before refilling. This would take an additional 7–8 days. The duration of the mandatory drainage in February was adjusted to weather conditions, with the aim to complete the refilling within the first week of March. Ice and snow cover could be tolerated, but exposure of sediments to air during extensive periods of frost should be avoided to minimize copepod resting egg mortality (Næss, 1991b).

A proxy for resting egg abundance in the sediment was estimated by hatching copepod nauplii from samples of sediment. Six 6.6 cm² and 0.5 cm thick surface sediment samples were collected with a plastic tube from the 500 m² mud flat next to the concrete dam with the plankton filter platform (Fig. 1b), when this area had been exposed to air for 2.5–3 weeks during draining. This area corresponds to between 1 and 1.5 m water depth when the pond is

filled. For removal of large particles, the samples were stirred in 0.5 L of 35 psu seawater and washed through plankton net sieves of 1000 and 250 μm, respectively. Then the samples were rinsed in a 40 μm sieve, and the remains on this sieve were incubated in glass jars with 0.5 L of 35 psu seawater at 23 °C with both 24 h roof lighting (fluorescent tubes) and outdoor lighting through a window. Hatched copepod nauplii were removed and enumerated on days 2 and 4 after incubation.

After refilling Svartatjern, inorganic granulated agricultural fertilizer (22-3-10 NPK, Yara ASA, Porsgrunn, Norway: www.yara.com) was added regularly to increase and maintain phytoplankton production. This fertilizer was added to a conical 120 L tank situated next to the mixer on the raft. The fertilizer was slowly dissolved by a steady water flow supplied through the tank by an impeller pump set to work for a period of 5 h. Effluent water was directed down to the mixer at 2.5 m depth and spread in the pond. The amount of fertilizer was assessed by Secchi disc readings, trying to keep a visibility of 1.0–1.5 m depth which would secure enough light for net primary production in the whole water column of the pond. Fertilization was reduced or stopped when visibility decreased in the desired range, and vice versa. Also periods of bright sunlight or heavy cloudy weather were considered regarding the amount of fertilizer added a specific day, trying to avoid an overload of nutrients. Fertilizer was added 2–3 times a week. Totals of 99, 230, and 90 kg fertilizer were added in 2011, 2012, and 2013, respectively. Calculated from manufacturer's product information, 1 kg fertilizer will give a 0.34 μM increase in ammonium concentration of the pond water, and similarly a 0.31 μM increase in nitrate. The amount of fertilizer added, calculated as average number of kg/day during the 120-day long growth periods before and after mid-summer, was 0.27 and 0.58 (2011), 1.84 and 0.14 (2012), and 0.69 and 0.08 (2013), respectively. On the actual fertilization days, total nitrogen nutrients in the pond water were calculated to increase on

average between 1.7 and 2.5 μM, with maximums between 3.2 and 6.4 μM.

Although silicate previously has been provided to Svartatjern and other mesocosms for enhancement of diatom growth (Egge and Aksnes, 1992), field observations and laboratory experiments encompassing a number of copepod species have found that certain kinds of diatoms may deteriorate egg production and hatching caused by specific chemical constituents of these algae, e.g. polyunsaturated aldehydes (PUA) and oxylipins (Poulet et al., 1994; Ban et al., 1997; Chaudron et al., 1996; Uye, 1996; Miralto et al., 1999; Ianora et al., 2003, 2004, 2009, Ianora and Miralto, 2010). Despite some uncertainty about the potential impairment of diatoms on copepod reproductive success (Jones and Flynn, 2005; Jónasdóttir et al., 2011; Amin et al., 2011), silicate was not used as a specific fertilizer in Svartatjern.

The mixer speed was tuned down to the minimum where adequate oxygen levels were kept at bottom depth and no stratification occurred in the water column below the thin freshwater layer at the surface. To reduce the thickness of the freshwater layer, increase salinity, or change other hydrographical characteristics, seawater was pumped in from the fjord. Before 2001, Svartatjern was emptied and refilled yearly in July. Originally, the main reason was to be rid of an unidentified stalked protozoan ectoparasite that occurred in large numbers on the exoskeleton of the copepods in late June and July. Summer drainage was suspended in 2011 and 2012, and consequently this parasite was seen again in 2012. However, summer drainage was carried out in 2013. These procedures were similar as in February, and after 1–2 weeks air exposure and refilling in late July, new copepod generations quickly re-established from hatching of resting eggs. As temperature fell during the autumn, keeping high temperature in the pond water was facilitated by the thin freshwater layer. However, to provide the best survival of copepod resting eggs during winter quiescence in January (Holmstrup et al., 2006), cooling of the pond water was initiated in early December by tilting the mixer for breaking down the surface freshwater layer. Exact timing was deduced from evaluation of weather forecasts and actual conditions, particularly regarding rainfall and air temperature. During winter, the aim for the hydrographical conditions of the stagnant pond water was to keep salinity above 23 psu, and temperature between 0 and 4 °C. To be prepared for drainage in February, the mixer was taken out of water and the pump on the raft lowered to 0.5 m depth. This would ensure that drainage could start in February, even with ice cover on the pond. Lack of mixing would allow oxygen depletion of the water during the winter quiescence and possibly anoxia to build up in the bottom water and sediment.

2.5. Hydrographical and biological sampling

Twice a week through the production season, hydrographical measurements, Secchi depth, and biological sampling were carried out. Hydrographical data (temperature, salinity and oxygen saturation) were collected between 09:00 and 13:00 on the raft (Fig. 1c) at 0, 0.5, 1, 2, 3, 3.5, and 4 m depths by a handheld WTW 310i multi-parameter instrument with ConOx-6 combination electrode for oxygen, temperature, and conductivity (WTW GmbH, Weilheim, Germany: www.wtw.de). Secchi depth was measured with a white disc (Ø = 300 mm) in the same position, as was collection of zooplankton for abundance estimation. Zooplankton was first collected by a 12.2-L Schindler-Patalas trap (Schindler, 1969) until 30th September 2011, when it was replaced by a 11.7-L tube sampler. The tube sampler was a 2.32 m long PEH pipe (Ø = 80 mm inside) with a valve on top. The tube was quickly lowered into the pond water in vertical position and the valve closed when the tube was filled. The enclosed body of water was filtered through a 60 μm plankton net sieve and fixed in a 1:50 Lugol's solution. A comparison between the two plankton sampling devices used gave an estimate of 3.8% less plankton numbers with the tube compared to the Schindler trap. The tube seemed to collect copepod nauplii more efficiently (17.9%), while the Schindler-Patalas trap was better for the copepodids and adult copepods (18.9%). Among three replicate tube samples, coefficient of variation was 8 and 36% for nauplii and copepodids, respectively. Since the target of the plankton collection was nauplii, and the tube sampled several depth strata, further sampling was carried out with the tube.

The samples were stored dark at 4 °C and enumerated after flushing with fresh water in a 40 μm sieve. To obtain a reasonable counting density (minimum 100 individuals of the most common plankton category), a plankton splitter (Motoda, 1959) was used to a maximum of 5 rounds when necessary. Counting and identification was carried out with a Leica MZ75 stereo microscope (Leica Microsystems GmbH, Wetzlar, Germany: www.leica-microsystems.com), usually at 25× magnification.

3. Results

3.1. Hydrographical data

After filling Svartatjern in March, salinity was between 30.5 and 32.5 psu which reflected the variability at 35 m depth in the nearby fjord at that time of the year. A steady drop in salinity occurred throughout the production season, ending up in the range of 24–25 psu in late November in 2011 and 2012 (Fig. 3a). In 2013, salinity was 33 psu at refilling in July, declining to 27 psu in late November. Salinity was increased several times by pumping in seawater during the production seasons (Fig. 3a).

At the start in March, temperature increased from an initial range 3–7 °C to a plateau of 15–20 °C at the end of May (Fig. 3b), increasing further to 20–24 °C in June and July. For the two years with no summer drainage (2011 and 2012), the temperature decreased steadily from late August, to about 8 °C in late November. In 2013 when Svartatjern was drained in July, a new initial temperature of 17 °C was measured at the time of completed refilling. The temperature then increased to 22 °C by the end of August and subsequently declined to late November in accordance with the two previous years.

Oxygen saturation increased rapidly after pond refilling in March (Fig. 3c), peaking at 200–260% in April and May. From June to August, oxygen saturation varied between 90 and 220%, declining slightly before it fell quickly in mid October to levels below 60%. Secchi disc readings showed that visibility declined all three years from 4.5 m (bottom) at filling in March to between 2.6 and 1.4 m in May (Fig. 3d). In 2011 and 2012, visibility was between 0.6 and 2.1 m from June until late October, before it increased quickly to more than 4 m in November. In 2013, a technical incident with the fertilization pump made it impossible to add fertilizer between late August and mid October, resulting in visibility in the pond remaining above 3.5 m during that autumn.

3.2. Copepod species and abundances

The abundance of copepod resting eggs in the sediments was not determined in February 2011. However, very few copepod nauplii or copepodids were observed in the pond water during March and April that year. A slight increase in copepodids seemed apparent in late May, but the plankton was not counted before a sampling programme was established in mid-June. Low abundances of copepod nauplii persisted until increasing rapidly in late July. Total densities of copepod nauplii peaked between 40 and 50/L several times from August to October, and increased further to a maximum of 90/L in November (Fig. 4a). Peaks in densities of copepodids were seen
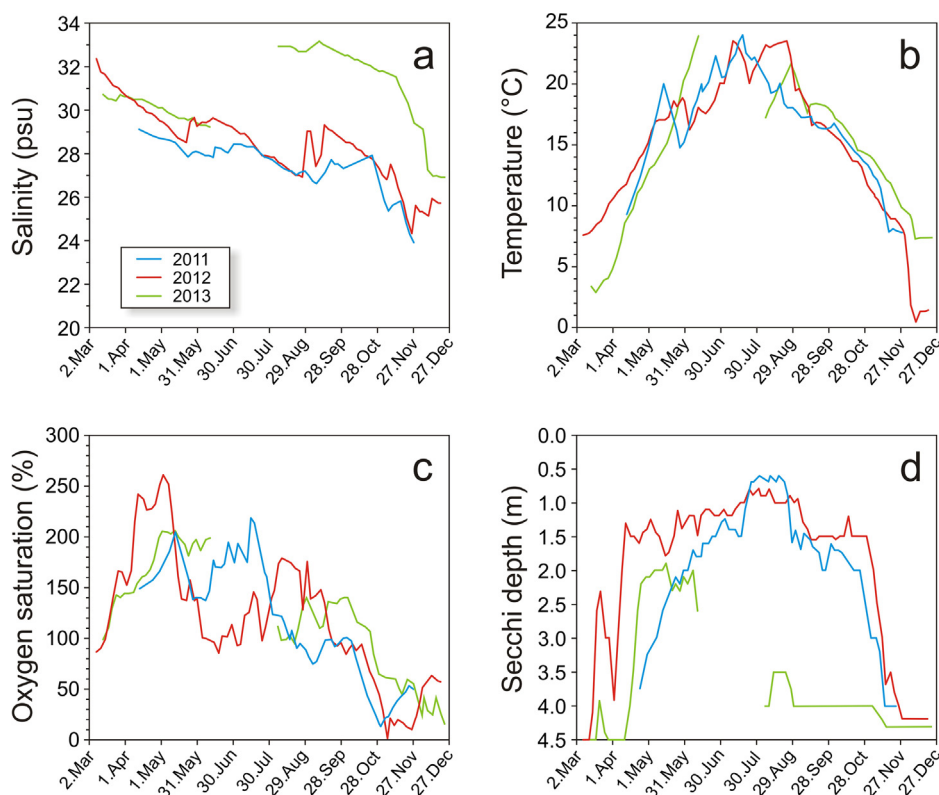
Fig. 3. Hydrographical data and visibility in the centre of Svartatjern from three years of operation after the reconstruction of the pond.

in mid-August, early October, and November, with maximums of 110 and 98/L for stages CI-CV and CVI, respectively. Cyclopoid and harpacticoid copepods occurred in low numbers (<12/L) throughout 2011, and miscellaneous zooplankton like cladocerans, rotifers, gastropods, bivalves, and polychaets were observed, but rarely exceeded 10/L. Of the calanoid copepods, *Centropages hamatus* (Lilljeborg) and *Acartia longiremis* (Lilljeborg) were identified as the most abundant species (Fig. 4b), but also *Acartia discaudata* (Giesbrecht) was observed in some quantities.

In February 2012, incubation of sediments confirmed occurrence of resting eggs. An average density of 43.8 viable eggs/cm^2 ± 23.9 (SD) was found from counts of hatched nauplii. This corresponded to a total of 4.6 ± 2.5 billion resting eggs in the pond, assuming an even distribution of the eggs on the sediment area. Shortly after refilling in early March, copepod nauplii peaked at 239/L, followed by successive abundance elevations of copepodids CI-CV (149/L) and copepodid CVI (63/L) (Fig. 4c). From mid April to end of May copepod nauplii varied between 87 and 252/L, and then dropped below 83/L until end of August. An increase in copepod nauplii abundance occurred during autumn, with a maximum of 177/L in mid-September, but declined to 16/L in November. However, copepodid densities remained low during this period and did not exceed 33 and 11/L for stages CI-CV and stage CVI, respectively. In 2012, *A. longiremis* was the dominating species among the calanoid copepods (Fig. 4d), and cyclopoid and harpacticoid copepods never exceeded 9/L. No new copepod species were detected in 2012. Miscellaneous zooplankton was below 8/L until late September but increased during October to a maximum of 66/L due to a bloom of polychaet larvae.

In February 2013, the resting egg abundance was determined to 432/cm^2 ± 164, giving an estimate of 45.4 ± 17.3 billion resting eggs in the pond. Hatching of the resting eggs immediately after refilling led to copepod nauplii densities above 161/L, with a peak of 584/L at onset of April (Fig. 5). From April, copepod nauplii abundance

declined to 42/L at end of June when summer drainage was initiated. Copepodids and adult copepods followed the same pattern as the nauplii, but with a delay resulting in a peak of 529/L in last half of April. After refilling in August, copepod nauplii and older copepod stages never exceeded 72 and 85/L, respectively (Fig. 5). Copepods were not identified to species in 2013, but both *Centropages* sp., *Acartia* sp., and harpacticoids were observed. In particular harpacticoids were seen during the autumn of 2013. Bivalve larvae, most likely the common European cockle (*Cerastoderma edule* L.) and the blue mussel (*Mytilus edulis* L.) that both were observed in the pond, bloomed during April 2013, but were not enumerated.

3.3. Copepod harvest

Copepod harvest was carried out in both 2012 and 2013 (Fig. 6), and added up to a total of 2.6 and 1.6 billion individuals over 40 days in 2012 and 61 days in 2013, respectively. Plankton filtration was done in periods of 3.0–16.0 h/day (2012) and 2.5–13.3 h/day (2013). The filtration efficiency, given as number of copepods in any stage collected per hour for transport to the larval fish hatchery (Fig. 6), was on average $7.0 \times 10^6 \pm 5.9 \times 10^6$ for 2012 and $4.8 \times 10^6 \pm 3.2 \times 10^6$ for 2013.

Damage from wheel filter filtration to the copepods was assessed to be less than 2% on copepodids when comparing physical damage on copepods found in the sedimented material in the collection tanks with the total amounts of copepods collected in the these tanks.

4. Discussion

Copepod production techniques have diversified by a number of methods ranging from outdoor extensive production in ponds to high-density indoor intensive systems under controlled environments (van der Meeren and Naas, 1997; Støttrup, 2003;
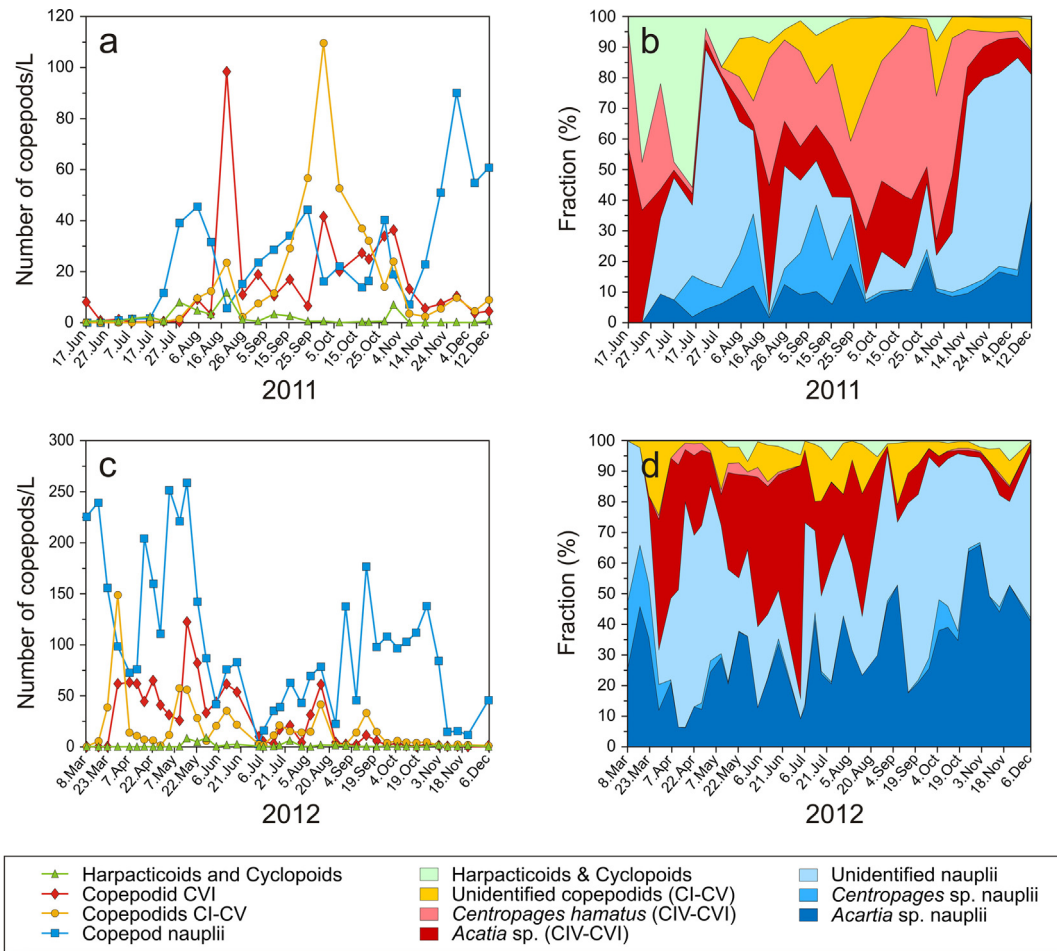
Fig. 4. Copepod abundance with species and stage distribution in Svartatjern during the two first years of operation after the restart in 2011.

Engell-Sørensen et al., 2004; Ogle et al., 2005; Drillet et al., 2011a).
The seawater pond Svartatjern belongs to the extensive approach
and has been successfully used in 20 of the last 30 years as a
provider of high-quality copepods (van der Meeren et al., 2008) for
a variety of scientific studies with larval marine fish (e.g. Kjørsvik

Fig. 5. Abundance of copepod nauplii and copepodids in Svartatjern in 2013.

et al., 1991; van der Meeren, 1991; van der Meeren and Næss,
1993; Næss et al., 1995; Conceiçao et al., 1997; Suthers et al., 1999;
Finn et al., 2002). Marine or brackish water ponds or mesocosms
producing copepods by the extensive method are most commonly
explored or used in tropical to temperate regions (Lee et al., 2004).
In contrast, Svartatjern is a high-latitude mesocosm system that
can deliver mainly calanoid copepods up to 8 months a year. The
advantage of a large-sized pond is its ability to supply substantial
amounts of copepods of any stage over a prolonged period when
high-quality prey for larval marine fish is needed. This can even
be the case when copepod densities are low, because in such large
systems copepod harvest is a matter of filtering capacity and effi-
ciency. For example, after the restart in 2012, Svartatjern supplied
successfully all the live prey, both copepod nauplii and copepo-
dids, in a nutritional study of initially 300,000 Atlantic cod larvae
(*Gadus morhua* L.) over a 40-day period from initiation of exogenous
feeding until the larvae were weaned on a formulated diet.

The copepod production in Svartatjern is carried out in a com-
plete and natural ecosystem where manipulation of the pond keeps
the copepods as top grazers or predators of the food web. Here,
the copepods may feed on a natural assemblage of autotrophic
algae, heterotrophic flagellates, and protozoans like various types
of ciliates. The assemblages of single-celled organisms have shown
high diversity with regularly high densities of microalgae in the
size range 3–5 μm equivalent spherical diameter before the pond
was closed in 2001 (Fig. 7). Ciliates were occurring frequently
(Naas et al., 1991), with averages between 40 and 60 ciliates/mL
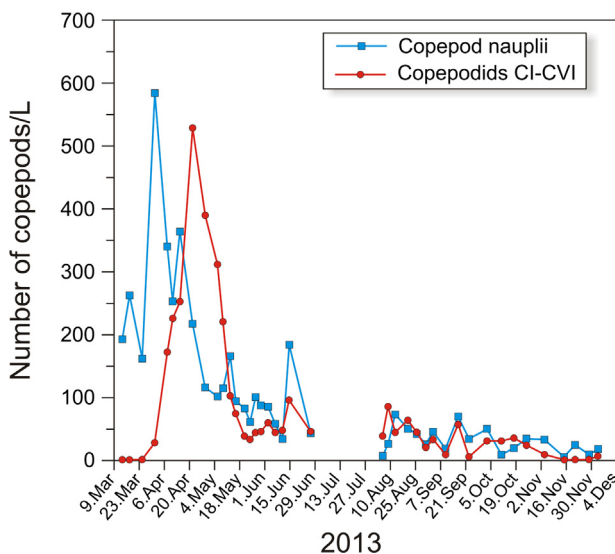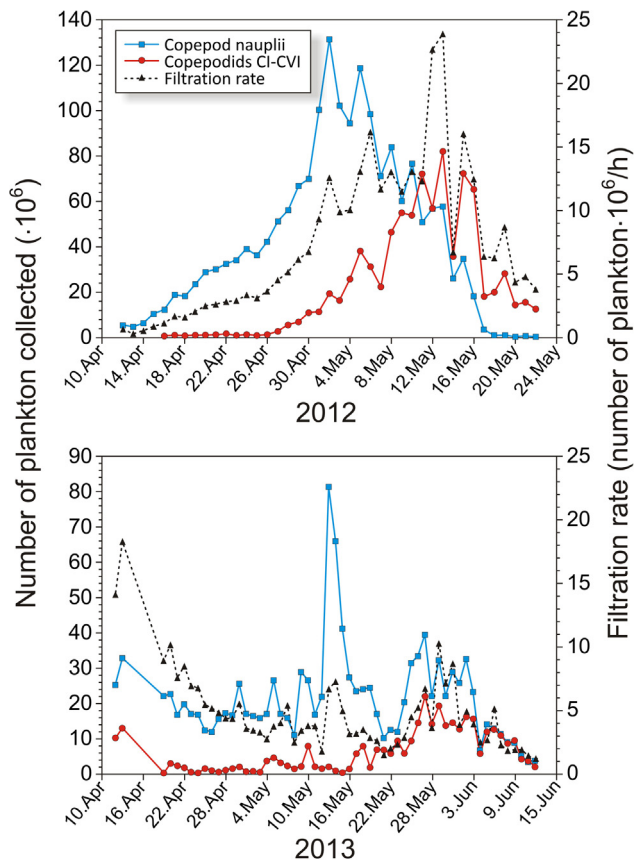over a year of weekly sampling (Fig. 7). The lower-trophic-level

Fig. 6. Amounts of copepods harvested and filtration rate of the plankton filter system during spring season 2012 and 2013.

plankton communities in Svartatjern should therefore ensure a highly diverse and sufficient food source for the copepods in the pond which in turn may enhance copepod growth and egg production rates by selective feeding and optimal nutrition (Klein Breteler, 1980; Stoecker and Egloff, 1987; Wiadnyana and Rassoulzadegan, 1989; Turner and Granéli, 1992; Ohman and Runge, 1994; Kleppel and Burkart, 1995; Milione and Zeng, 2007; Camus et al., 2009; Dhanker et al., 2013). Optimal nutritional status of the copepods in Svartatjern has indeed been verified (van der Meeren et al., 2008). However, despite apparently high algal production as indicated
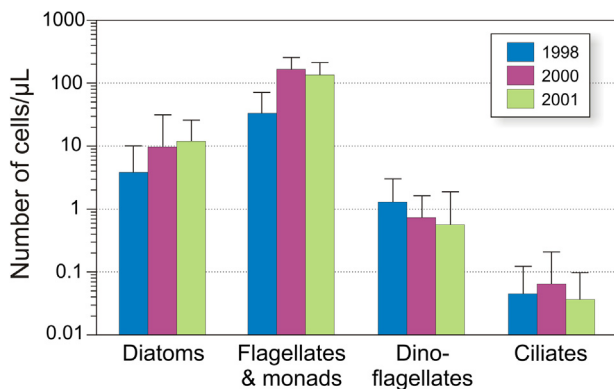

Fig. 7. Average abundance of main phytoplankton groups and ciliates among weekly samples from 1 m depth in Svartatjern from March to October (season of net primary production) during the years 1998, 2000, and 2001 (data from samples fixed in pseudo-Lugol, Verity et al., 2007). Note the logarithmic ordinate axis. Between 91 and 98% of the flagellates and monads were unidentified and in the size range 2–5 μm equivalent spherical diameter.

from previous Svartatjern data (Fig. 7), oscillations in copepod densities show that there are traits of copepod feeding, reproduction, and life history that are not yet fully understood in this system. For example, a number of copepod species have been documented to be omnivorous feeders that also can prey on copepod eggs and nauplii (Landry, 1978; Conley and Turner, 1985; Daan et al., 1988; Hada and Uye, 1991; Lazzaretto and Salvato, 1992; Uye and Liang, 1998; Boersma et al., 2014), and the regulatory force of such predation should be included in future studies of copepod dynamics in Svartatjern or similar systems.

In 2012 and 2013, the copepod production in Svartatjern during the spring season was clearly linked to occurrence of resting eggs in the sediments, as the copepod nauplii abundances peaked shortly after refilling in early March. The nauplii abundances in March were also three times higher in 2013 compared to 2012, when amounts of nauplii hatched from resting eggs in the sediment samples were ten-fold higher than in 2012. No such early naupliar peak was observed in 2011 when the pond was refilled after 10 years of rest. Also the smell of sulphide was prominent when the10-year-old seawater was removed from the pond during February 2011. This indicates that a thick freshwater layer on top of a long-lasting anoxic sulphide-rich seawater layer probably killed all remnant resting eggs in the sediments from the production periods before 2002. Of the three previously dominating copepod species, *Eurytemora affinis* (Poppe) and *Paracartia grani* (Sars) did not reoccur after the 10-years rest period, while *C. hamatus* again was observed in 2011 along with new *Acartia* sp., predominantly *A. longiremis*. Only a one-year production season was necessary to prime the pond with enough resting eggs for use as a predictable source of copepod harvesting. Under favourable feeding and environmental conditions, copepods produce subitaneous eggs that will hatch within days. In addition, calanoid copepods species may produce resting eggs when approaching unfavourable conditions, including *A. longiremis* and *C. hamatus* (Alheit et al., 2005; Marcus and Lutz, 1998). Resting eggs comprise three types of dormancy, from quiescence (retarded development), oligopause (delayed hatching), to diapause (arrested development) (Grice and Marcus, 1981; Dahms, 1995; Marcus, 1996; Chen and Marcus, 1997; Marcus, 2006; Drillet et al., 2011b). Diapause eggs may have extreme longevity and have been found viable after about 70 years in sediments of good conditions (Dahms et al., 2006; Sichlau et al., 2011). Resting eggs are a common life history characteristic of many neritic calanoid copepods, and such eggs occur frequently at high densities in the sediments of Norwegian marine enclosures, lagoons, and ponds (Næss, 1991a, 1996). These eggs will sink to the bottom and are tolerant to adverse environmental conditions like large temperature fluctuations, desiccation, freezing, anoxia, and sulphide exposure, as well as exposures to various chemicals like disinfection agents (Næss, 1991a, 1991b; Næss and Bergh, 1994; Marcus and Lutz, 1998). Also subitaneous eggs seem to have mechanisms to resist detrimental effects of anoxia and sulphide for some period (Nielsen et al., 2006). Copepod species that produce resting eggs are ideal to high-latitude enclosure or pond rearing. These eggs will survive draining during both winter and summer which may be carried out to prevent establishments of nuisance organisms. It is not clear whether the resting eggs in Svartatjern are quiescence, oligopause, or diapause eggs, but copepod eggs with spiny surface structures resembling those described by Castro-Longoria (2001) for *Acartia tonsa* (Dana) diapause eggs have been observed in the pond. However, surface structures may not be used as an indicator of egg type since Hansen et al. (2010) described 4–5 varieties of similar spiny surface structures on *Acartia* spp. and *C. hamatus* subitaneous eggs. Næss (1991a) suggested that the overwintering resting eggs in Svartatjern most likely were diapause eggs because of their longevity and tolerance. On the other hand, the synchronous hatching may indicate that the resting

eggs in Svartatjern are quiescence eggs. Efforts to develop secure methods for determination of egg types in copepods are clearly needed.

Based on 20 years experience in Svartatjern, a seasonal management protocol has been developed where cooling and stop in mixing are initiated in December for enhancement of resting egg winter survival, followed by drainages in February and July for control of nuisance organisms. The second draining during summer was for various reasons not carried out in 2011 and 2012, with the result of blooms of polychaet and bivalve larvae during fall 2012 and spring 2013, respectively. In December, controlled cooling to 1–4 °C is carried out by adjusting the mixer's angle and speed, followed by a complete stop in mixing during January with oxygen depletion and sulphide formation in the sediments and in the water layers over the bottom in the deep parts of the pond. Such conditions may be beneficial for the resting eggs, and the anoxic layer may also reduce the life conditions for nuisance organisms. The combination of low temperature (4–5 °C) and plausibly low oxygen or anoxic conditions have been shown to prolong the survival of copepod eggs stored in sediments when compared to storage at higher temperatures (Uye, 1980; Ban and Minoda, 1992). Diapause eggs of *C. hamatus* have been found to survive as long as 437 days at ambient field temperatures in anoxia compared to normoxia (Marcus and Lutz, 1998). Furthermore, anoxic conditions have promoted greater accumulation of viable *A. tonsa* eggs at the sediment surface than in normoxic treatments (Scheef and Marcus, 2011). Storage conditions with temperatures below 5 °C, medium salinities, and anoxic conditions have been found most optimal for *A. tonsa* quiescence eggs (Holmstrup et al., 2006). Following the procedures of the management protocol led to high densities of viable resting eggs in the Svartatjern sediment in February 2013, comparable to the highest resting egg abundance observed in Norwegian ponds and enclosures (Næss, 1996). Temperatures below 1 °C during the chilling period (Fig. 1b) did not seem to harm resting egg viability in the pond. Draining of the pond in February will expose the sediments and the eggs to combinations of rain, snow, frost, or sun for a period of some days (in the centre) to 3–4 weeks (in the shallow areas). Refilling implies a 3–5 °C increase in temperature and elevated oxygen saturation to near 100%. These changes happen a few weeks before spring equinox when the daily change in photoperiod is approaching its maximum and becomes consistent with a 12L:12D cycle. Although the mechanisms for hatching of resting eggs in Svartatjern are not clear, hatching of diapause eggs has been synchronized in relation to fluctuations in temperature and photoperiod (Marcus, 1996; Boyer and Bonnet, 2013). Other cues like oxygen concentration have also been suggested as a regulatory mechanism (Uye and Fleminger, 1976; Dahms, 1995). Eggs collected from the Svartatjern sediment in late February were easily hatched within 2–4 days in the laboratory under even more extreme changes of the environment when brought from ambient outdoor conditions to indoor with 23 °C, 100% oxygen saturation, and 24 h roof light with an additional 11L:13D cycle signal by natural light through a window.

Use of semi-natural copepod production in large enclosures may introduce a potential risk for transfer of diseases or parasites (Su et al., 2006; Drillet et al., 2011b). Use of zooplankton from large enclosed lagoons in Norwegian marine aquaculture has given infections of helminth parasites in Atlantic halibut (*Hippoglossus hippoglossus* L.) larvae (Bergh et al., 2001). Similarly, the digenic trematode *Cryptocotyle lingua* (Creplin) which uses the gastropod *Littorina littorea* (L.) as intermediate host, have been observed in copepod-reared turbot (*Scophthalmus maximus* L.) larvae (unpublished data). However, during the 20 years that Svartatjern has been used as a copepod pond for larval fish experiments, infections of fish parasites or pathogens has not been traced back to the pond, though measures should be taken to monitor this in the future. In contrast to the large enclosed lagoons, Svartatjern can be drained completely. Further, the copepod production is based on resting eggs within the system and not collection of wild plankton. In these ways, intermediate hosts will not be allowed to establish in the pond, which may explain the good record of Svartatjern so far.

Extensive production in ponds or large enclosures generally entails large numbers of copepods at moderate to low densities (Ogle et al., 2005). Thus, because of the large volume of such systems, the filter capacity will limit the amount that can be harvested. In Svartatjern, the harvest rate estimated from the pump capacity was calculated to 0.24% of the pond volume per hour of filtration, which corresponded to an actual daily filtration rate in the range of 0.7–3.8% of the total pond volume in 2012 and 1.2–2.4% in 2013. Given an exponential decay model, it will take 19 and 99 days at the highest and lowest of these filtration rates, respectively, to remove 50% of the plankton in the pond. It should be noted that these filtration rates only represent the needs for live feed during specific start-feeding experiments with marine fish larvae. During early start-feeding, only nauplii are needed, and copepodids are continuously transferred back to the pond without being registered in the harvest. Further, improvements in filtration technology can clearly improve the daily yield from Svartatjern. The question is how much can be filtered per day without halting the copepod production? Copepod generation time will vary with temperature and feed conditions, and harvesting rate should be lower than reproduction rate. Maximum yield of *Acartia tsuensis* (Ito Tak) from 24 m³ outdoor tanks at 24–28 °C and chlorophyll-*a* levels of 10 µg/L was achieved when 15 to 30% of tank volume was harvested daily (Ohno et al., 1990). Better reproductive abilities were achieved at lower population densities, and higher population densities resulted in an extension of development time for the last naupliar stage and all the five copepodid stages that were not caused by food limitation. Further, a 10% daily harvest rate of *Eurytemora affinis* from 25 m³ outdoor tanks at temperatures in the range 15–20 °C gave stable levels of nauplii abundance when the removed water was replaced by new seawater or algal cultures (Nellen et al., 1981). However, when the copepods were harvested by nets and no water was replaced, the nauplii abundance decreased. Considering these results and the chemical mediated switch to diapause egg production in *Eurytemora affinis* at high population densities (Ban and Minoda, 1994), factors like periodic water renewal, increased filter capacity, and harvest rate might be beneficial for the stability of the copepod populations in Svartatjern. Diapause egg production is primarily initiated by changes in photoperiod and temperature as cues for signalling commence of environmental adversity for a particular copepod species (Dahms, 1995; Hairston and Kearns, 1995), but also crowding and insufficient feed conditions have been shown to induce diapause egg production in calanoid copepods (Ban and Minoda, 1994; Drillet et al., 2011b). Details of these mechanisms would enable controlled resting egg production prior to drainages of the pond. To optimize copepod culture and harvest in a large enclosure like Svartatjern, more knowledge is needed about copepod population dynamics and life strategies at species level under various culture and harvest conditions.

In conclusion, an enclosure like Svartatjern may provide all stages of copepods in adequate amounts for large start-feeding experiments with larval marine fish, and where the nutritional requirements are of most importance. The copepods from such enclosures could also be used by commercial hatcheries, at least for supplement of nutritionally high-quality live prey during critical periods of larval development (Næss et al., 1995). Due to the large enclosure size, potential limitations in harvest caused by fluctuations of the copepod abundance in Svartatjern can be overcome by adjusting the daily filtration rate. Further, the seasonal changes in light and temperature make the copeod production in Svartatjern highly dependent on resting eggs. This has enabled a seasonal

management protocol that aims at minimizing establishment of nuisance organisms by pond drainage twice a year, followed by synchronous hatching of copepod resting eggs surviving in the sediments. Future research to optimize plankton production and harvest should focus on mechanisms that affect copepod population dynamics and production of resting eggs, including their environmental requirements for optimal dormancy, survival and hatching. As copepods are delicate animals, development also of a more efficient and gentle filtration system could further increase the harvest rate and benefit survival.

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