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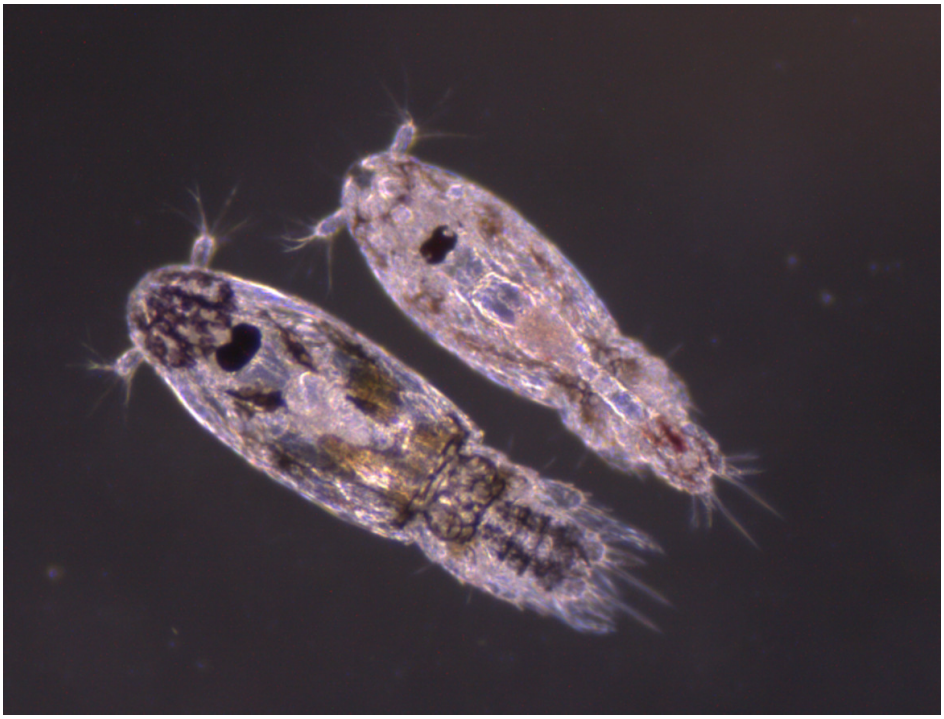
Eirikur Danielsen



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Frágreiðing

Heiti

Identification of the free swimming larval stages of *Caligus elongatus* and *Lepeophtheirus salmonis*

Høvundar

Eirikur Danielsen

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Samandráttur

This report describes a methodology to identify *Lepeophtheirus salmonis* and *Caligus elongatus* at the nauplius I, nauplius II and copepodite stages by morphological characteristics.

Leitorð

Laksalús (*Lepeophtheirus salmonis*), Seiðalús (*Caligus elongatus*), Nauplius I, Nauplius II, Kopepodit (Copepodid/Copepodites)

1. Introduction

Sea lice, especially *Lepeophtheirus salmonis* and *Caligus* spp., have the greatest economic impact of any parasite in salmonid fish farming (Costello, 2006). In the Faroe Islands, the two species *Caligus elongatus* and *Lepeophtheirus salmonis* are considered an increasing threat to the salmon (*Salmo salar* L.) farming industry. A key in resolving this issue is more research on the small and large scale spatial and temporal distribution of the free swimming nauplii and the copepodites around the islands. As part of this research, identification of the two species and their stages is essential. However, due to the tendency of the species to look analogous until near adult, it is difficult to differentiate between the two species at their larval stages (Galbraith, 2004). This report describes a methodology to identify the two species at the nauplius I, nauplius II and their copepodite stage by means of morphological differences.

2. Preservation

Identification must take place quickly after sampling, as the pigmentation of the nauplii and copepodites vanish after only a few days. The two species are very similar in shape and structure, and species identification without pigmentation characteristics can be very difficult.

Tests of comparison between specimens preserved in ethanol (96%) after fixation in formaldehyde (8-10%) for a day, and specimens preserved in formaldehyde (8-10%) only, have yielded much better results for ethanol-preservation. After only four days, a clear difference was detected in the pigmentation, as well as in the clarity of the eyes of *L. salmonis* copepodites. The specimens preserved in formaldehyde only had much more transparent eyes, and were less clearly pigmented than those preserved in ethanol. After seven days most of the pigmentation was gone, and the eyes of the ones preserved in formaldehyde only were even more transparent (Fig.1).

Since this deterioration takes place, it is recommended to preserve the samples in formaldehyde on the day of sampling only, and then preserve

them in ethanol for the remaining period. All samples were stored at room temperature.

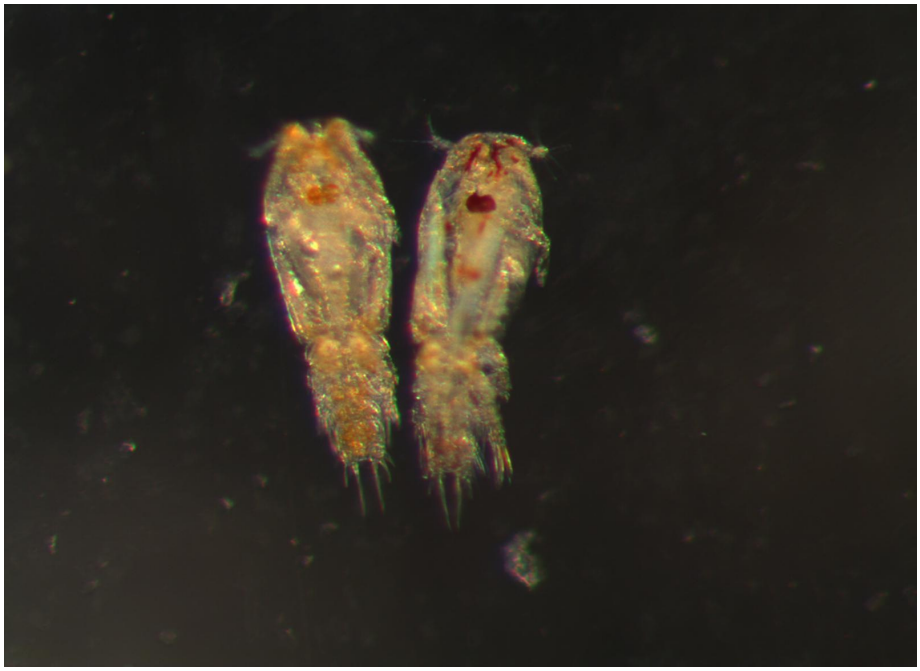

Fig. 1 *L. salmonis* copepodites preserved in formaldehyde (8-10%) (left) and ethanol (96%) (after fixation in formaldehyde (8-10%) for a day) (right) for a period of seven days.

3. Identification

At 7°C it takes about 6 days for salmon lice (*L. salmonis*) to develop from the nauplius I stage to the copepodite stage. At 12°C the same transformation only takes 3 days (Heuch et al. 2000). The duration of the first nauplius stage is relatively short (Table 1) and therefore it is more likely to find either the nauplius II or the copepodite stage when sampling, especially at high temperatures.

Table 1 Duration (hrs) at the nauplius I and II stages of *L. salmonis* and *C. elongatus* at temperatures 5, 10 and 15 °C and average length and width (mm) of the two nauplii stages of *L. salmonis* (Johnson & Albright, 1991, Pike et al. 1993, Schram, 1993). The nauplii of *C. elongatus* are slightly smaller than those of *L. salmonis*, but their size range might overlap.

Species	Stage	Temperature (°C)	Duration (Hrs.)	Length (mm)	Width (mm)
L. salmonis	Nauplius I	5	52	0.5	0.2
		10	30.5	0.5	0.2
		15	9.2	0.5	0.2
L. salmonis	Nauplius II	5	170.3	0.6	0.205
		10	56.9	0.6	0.205
		15	35.6	0.6	0.205
C. elongatus	Nauplius I	5	36.9		
		10	27.6		
		15	16.6		
C. elongatus	Nauplius II	5	159.1		
		10	68.1		
		15	41.1		

The anterior end of the nauplii of *L. salmonis* and *C. elongatus* is wide and gently rounded, whereas the posterior end is narrow and blunt. Additionally the nauplii of both species are equipped with two appendages posteriorly, which protrude laterally on each side (Schram, 2004). What differs most between the two species is their pigmentation. At all developmental stages *L. salmonis* is slightly larger than *C. elongatus*, especially the copepodites. The size range of both species, however, might overlap.

The *C. elongatus* and *L. salmonis* nauplii can be confused with Euphausiid nauplii, because they have no mouth, two setae which function as stabilizers at the posterior end, and the same number of swimming appendages. The Euphausiid nauplii are, however, more circular in outline with a single eyespot compared to the two eyespots of the Caligid nauplii. Additionally, the setae of Euphausiid nauplii are short and spine-like rather than long and fleshy as those of the Caligid nauplii (Galbraith, 2004).

3.1 Nauplius I stage

At the stage immediately after hatching, the nauplii of both species are more rounded than at their second stage. For *L. salmonis* the nauplii are almost translucent, but black pigments are present at both the anterior and posterior end of the body. Black/brown pigments are found approximately in the middle of the body, distributed symmetrically on both sides of the intestine, and all appendages are unpigmented (Schram, 1993). The pigments are visible already before hatching, while the nauplius still is inside the egg, as well as immediately after hatching (Fig. 2). The pigments situated anteriorly are distributed around the eyes; however the eyes can be difficult to see.

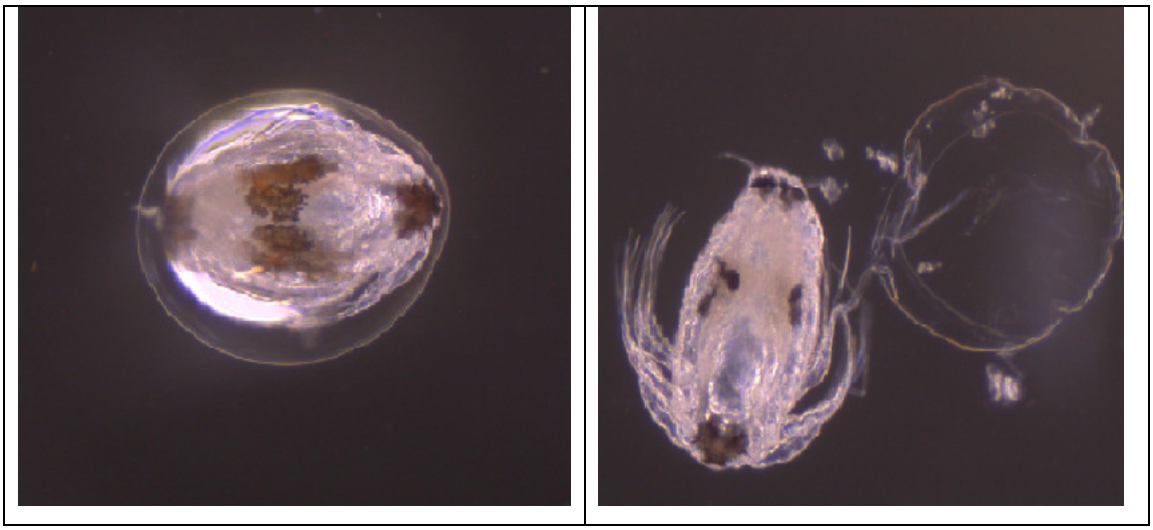

Fig. 2 *L. salmonis* nauplius I shortly before hatching (left) and shortly after (right).

At all stages, the pigments of *C. elongatus* clearly differ from those of salmon lice. Nauplius I of *C. elongatus* has red pigments rather than black. They are distributed along the side of the body, but mainly as two patches in the middle of the body when viewed dorsally (Fig. 3). Red lines appear on both sides of the body from the two patches, back to the posterior end, where a band of red pigmentation occurs across the body. Red pigments are also located at the anterior end around the eyes, though the eyes themselves can be difficult to see (Fig. 3).

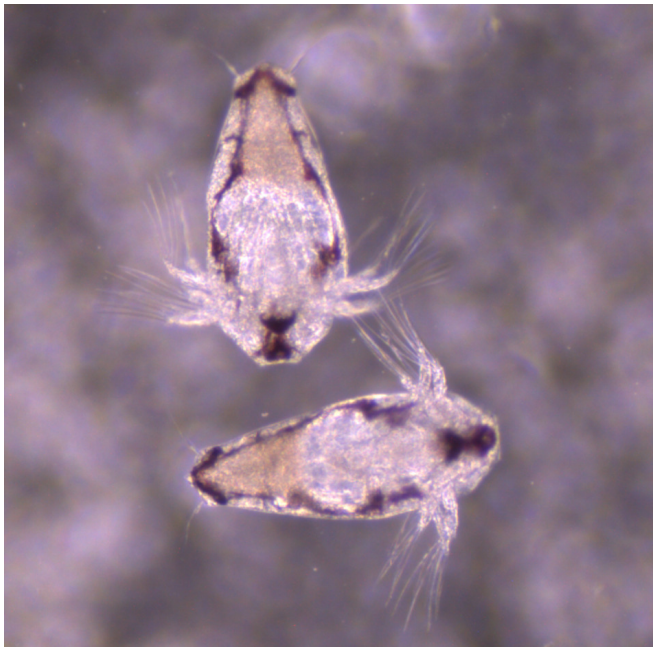

Fig. 3 ***C. elongatus*** **nauplii I.**

3.2 Nauplius II stage

The second stage nauplii are more oval and slender while their pigmentation is roughly the same as for the first stage of both species. The central body pigmentation of *C. elongatus* is, however, now divided in three pairs distributed along the body, which differ between individuals (Schram, 2004) (Fig. 4).

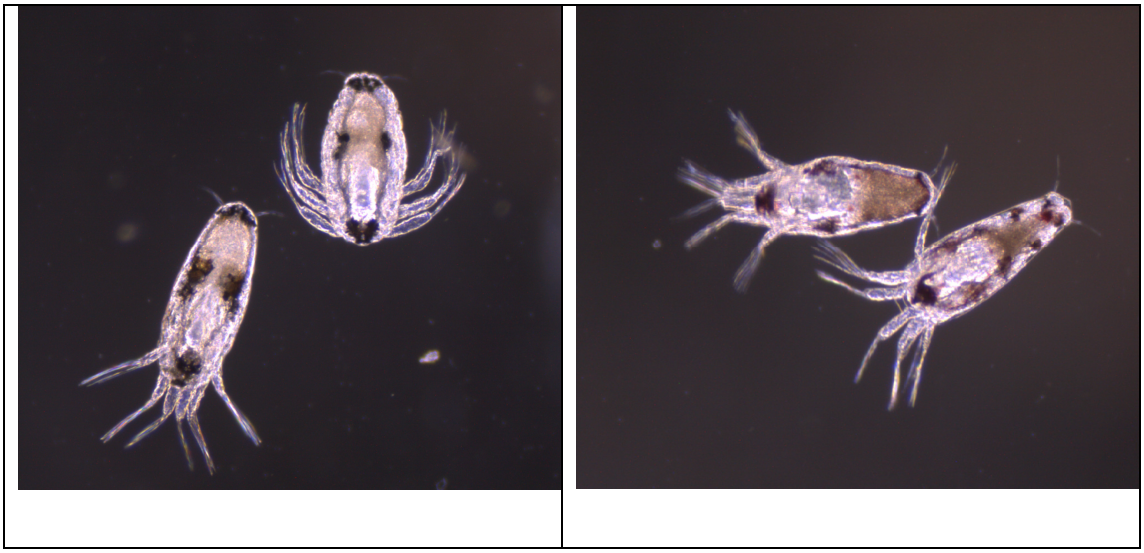

Fig. 4 Nauplius I and II of ***L. salmonis*** **(left) and** ***C. elongatus*** **(right). Nauplius I is the one above, while nauplius II is below.**

At the second nauplius stage *C. elongatus* is slightly more elongated and streamlined than *L. salmonis*, especially in the posterior body region (Fig. 5). The second nauplius stage of *L. salmonis* is a bit more rounded and bulky with two large symmetrically placed patches approximately in the middle of the body, most clearly viewed dorsally (Fig. 5). As the *L. salmonis* larvae grow older, the pigment of the copepodid urosome is visible through the naupliar cuticle as three to four bands across the larvae. These become progressively broader posteriorly (Fig. 6) (Schram, 2004).

Fig. 5 Nauplius II comparison, dorsal view (*C. elongatus* left, *L. salmonis* right).

Fig. 6 Older *L. salmonis* nauplius II.

The second nauplius stage of both species is equipped with an apical outgrowth centrally on the posterior margin (Schram, 2004). This, however, may be difficult to see, and is sometimes missing. The nauplius II larvae of *C. elongatus* have, in addition to the apical outgrowth, two posteriorly directed processes. These are on the posterior third of the ventral surface of the cuticle and these are hiding the precursors of the maxillipeds of the copepodites (Schram, 2004). These processes can be difficult to spot and other features are more recognisable when identifying *C. elongatus*.

3.3 Copepodite stage

The copepodites have two body parts. These are the non-segmented ovate prosome, which is the anterior body region and the four segmented urosome with caudal furca, which is a combined thorax and pseudoabdomen at the posterior body region. The swimming leg pairs are positioned as seen on Table 2. The third segment of the urosome (fifth thoracic segment) carries no appendages and the last segment is the undifferentiated genital complex (Galbraith, 2004).

Table 2. Position of the swimming leg pairs of the copepodites (Galbraith, 2004).

Swimming leg pair	*Position*
First pair	Second thoracic segment (part of the prosome)
Second pair	First segment of the urosome (third thoracic segment)
Third pair	Second segment of the urosome (fourth thoracic segment)

The copepodite stage of *L. salmonis* usually has more pigments and an overall darker colour than that of *C. elongatus.* Pigmentation of *L. salmonis* is dark with brown patches dorsally on the prosome and darker posteriorly on the urosome, as well as a black pattern anteriorly in front of the eyes. *C. elongatus* has a reddish colouration throughout the body, with patches along the body much like the second stage nauplii.

If only a few pigments remain and identification is difficult, the species can be differentiated by looking at the patches around the eyes, if these still remain. *L. salmonis* has two patches behind the eyes dorsally. These are two lines, one behind each eye, giving a "sad"-looking appearance (Fig. 7).

C. elongatus has a patch on each side of the eyes dorsally, and having the colour of a blush, this gives the copepodid a more of a "blushy"-looking appearance (Fig. 7). The position posteriorly where the prosome ends and the urosome begins is more clearly seen on the *L. salmonis* than on *C. elongatus*. Generally, the *L. salmonis* is larger than the *C. elongatus* copepodid (Fig. 7).

Fig. 7 Copepodite stage, dorsal view. *L. salmonis* left, *C. elongatus* right. *L. salmonis* has a black line behind each eye, whereas *C. elongatus* has a red patch on each side of the eyes.

4. Discussion & Conclusion

The pigments of individuals of both species and stages may vary. The shape may also vary, especially at the first nauplius stage where it is more rounded and short immediately after hatching, than later on.

The distribution and pattern of pigments dorsally on the prosome of *L. salmonis* copepodites varies, but the overall patchiness of anterior pigments in front of the eyes, the two lines right behind them, and the dark patchy urosome, are relatively constant. The two symmetrically placed C-shaped patches in the middle of the body of the first nauplius stage of *L. salmonis* are constant as well.

The pigments of *C. elongatus* copepodites vary individually as well. However, the symmetrically distributed red patches along the side of the body are reoccurring features. So are the two patches beside the eyes and red lines in

front of them on the anterior end of the body. The elongated shape and symmetrical distribution of the evenly spaced red patches on the side of the body of the second stage nauplii also appear to be constant, whereas at the first nauplius stage, the pigments appear as red lines along the sides rather than as actual patches.

It can be extremely difficult to distinguish between the first-stage nauplii of both species if much of the pigmentation is missing. The *C. elongatus* nauplius is, however, slightly more elongated and arrow shaped than the more oval *L. salmonis*. However, due to the osmotic pressure this difference in shape can be muddled if the samples are stored in seawater mixed with fresh water. It is therefore extremely important to preserve, store and handle the nauplii with care, and not allow fresh water to affect the nauplii during identification.

At the second nauplius stage the differences are more subtle, when excluding pigmentation. The shapes are somewhat similar, although *C. elongatus* is more elongated and slim-looking than *L. salmonis*. One way of comparison is to look at the difference between the widest point of the prosome and the narrowest point at the posterior end. At the second stage the difference in width between these two points is higher in *C. elongatus*, where the widest point is about twice as wide as the narrowest, whereas for *L. salmonis* the posterior end is a bit more than half the width of the widest body point. This may, however, vary between individuals of the same species, and thus should be taken into account alongside other features when identifying the two species.

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