A simplified method to estimate *Diphyllobothrium* spp. infection in salmonids

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Abstract

Some fish parasites constitute severe management problems as they may cause mortality of their fish host or are important zoonoses of humans. Parasite assessments are therefore critical in order to keep track of infections. If conventional sampling techniques can be simplified, parasite assessments might be easier to obtain, less time consuming and more extensive. In this study, we compare the assessed number of *Diphyllobothrium* spp. cysts (CYST) with the counted number of *Diphyllobothrium* spp. plerocercoid larvae recovered using a conventional digestive technique (LARV). The aim was to determine the potential of using CYST as a simplified methodology for assessing *Diphyllobothrium* spp. infection in salmonids. In total, 365 brown trout and 424 Arctic charr were sampled from nine lakes in subarctic Norway. Strong correlation, significant linear relationship and large amount of explained variation were found between $\log_{10}$CYST and $\log_{10}$LARV in both fish species. The method had a slight, but not significant tendency to work better in charr compared to trout. In addition, absolute difference between CYST and LARV increased at parasite intensities > 100 indicating that the method have reduced functionality when estimating parasite intensity in heavily infected salmonid populations. However, overall, by using this simplified and less time-consuming methodology, a good indication of *Diphyllobothrium* spp. intensity, abundance and prevalence was obtained. We suggest that this method provides a sound proxy of the *Diphyllobothrium* spp. burden and have the potential to be used in parasite assessment during fish monitoring and fisheries management surveys, particularly if the time and resources for detailed parasite studies are not available.

Introduction

Many fish parasites are ecologically important as they may affect the fitness of the host and induce host mortality (Esch 1994; Marcogliese 2004). Additionally, some might also be zoonotic and hamper the recreational value of their fish hosts, emphasizing the need for management initiatives (Knudsen, Amundsen & Klemetsen 2002; Torres et al. 2002; Chai, Darwin Murrell & Lymbery 2005; Dick 2007). Effective sampling methods and parasite assessment procedures are therefore needed in order to study such parasite populations, especially for management purposes. Parasite sampling and analysis is often a very time consuming and labor-intensive process. Large sample sizes might also be necessary as the distribution of these organisms can be strongly aggregated. In an effort to circumvent these issues, simplified sampling procedures might be a potential solution.

The endoparasitic cestode genus *Diphyllobothrium* has a cosmopolitan distribution and includes important zoonoses, emphasizing the need for distributional assessments (Dick, Nelson & Choudhury 2001; Chai et al. 2005; Scholz et al. 2009). In northern Europe, *Diphyllobothrium ditremum* and *D. dendriticum* commonly infect several salmonid species such as brown trout (*Salmo trutta* L.) and Arctic charr (*Salvelinus alpinus* (L.)), hereafter referred to as trout and charr, respectively. The infections of *D. ditremum* and *D. dendriticum* both result in the formation of large and easily detectable cysts on the stomach wall, within the viscera and sometimes also in the flesh of trout and charr. *Diphyllobothrium* spp. is transmitted to the salmonids through copepods as first intermediate host, or through fish as paratenic hosts (Vik 1964; Halvorsen 1970; Henricson 1977; Rahkonen & Koski 1997; Knudsen et al. 2008; Henriksen et al. 2016). When a salmonid fish eats an infected host (i.e.

copepod or fish), the parasite larvae penetrates the stomach of the predatory host and become encysted primarily on the stomach and intestine (Torres, Leyán & Puga 2012). In large piscivorous fish, these parasites can accumulate and reach very high intensities as infected fish may function as paratenic hosts (Curtis 1984; Knudsen & Klemetsen 1994; Kuhn et al. 2016; Siwertsson et al. 2016). High intensities of *Diphyllobothrium* spp. can cause fibrosis, necrosis, atrophy and inflammatory reactions in salmonids, causing them to lose their value for human consumption (Torres et al. 2002). *Diphyllobothrium* spp. infections might also induce host mortality, making knowledge and information about these parasites highly relevant in the management of salmonid populations (Bylund 1972; Henricson 1977; Halvorsen & Andersen 1984; Rahkonen et al. 1996; Hammar 2000).

The initial procedure of determining *Diphyllobothrium* spp. intensity in a fish host involved manually teasing every single plerocercoid larvae out from their cysts by the use of small needles (Meyer & Vik 1961). This technique, besides being extremely time consuming, was found to be unreliable as it ultimately underestimated the parasite infection (Meyer & Vik 1961). The current conventional procedure of quantifying *Diphyllobothrium* spp. infection in trout and charr involves an artificial digestion of infected tissue in order to excyst the live parasite larvae (Meyer & Vik 1961; Romeis 1968). Usually the stomach is dissected out from the host, emptied for content, and then placed, together with other cyst-containing tissues, in a digestive fluid mimicking the acidic stomach environment of the parasites´ avian final host. By keeping the sample at room temperatures for several hours, the live plerocercoids are excysted and can then be collected (Romeis 1968). The digestive technique is believed to recover close to 100 % of the actual number of live parasite and can therefore be seen as a very good indicator of the total *Diphyllobothrium* spp. infection in the host (Meyer & Vik

1961). However, when many fish are to be processed, even this technique becomes very time-consuming and labor intense, especially in heavily infected fish populations.

In this study, we explore the relationship between the assessed number of *Diphyllobothrium* spp. cysts and the counted number of *Diphyllobothrium* spp. plerocercoid larvae recovered using a conventional digestive technique. Brown trout and Arctic charr were sampled from several lake localities in northern Norway. The main objective was to explore whether an assessed number of *Diphyllobothrium* spp. cysts can be used as a simplified methodology for fish management surveys and certain scientific studies, where traditional sampling procedures are not feasible due to time and/or labor constrains. Hence, the principal hypothesis tested was that there is a strong and significant correlation between assessed number of *Diphyllobothrium* cysts and counted number of plerocercoid larvae in trout and charr.

Materials and methods

Sampled lakes

During August 2010-15, the salmonid populations of nine lakes in Northern Norway were sampled. The lakes included six lakes from Nordland county and three lakes from Troms county (see Table 1 for lake physical parameters and catch data). Knudsen et al. (2008), Sánchez-Hernández & Amundsen (2015) and Kuhn et al. (2015) provide additional information about the lakes. A diverse range of *Diphyllobothrium* spp. infections as well as

feeding ecology and piscivorous behavior in brown trout and Arctic charr is found among these sampling sites (Eloranta, Knudsen & Amundsen 2013, Sánchez-Hernández & Amundsen 2015, Henriksen et al. 2016, Kuhn et al. 2016).

Fish sampling and parasite recordings

Fish were caught by deploying multimesh survey gillnets (10 to 45 mm mesh size from knot to knot) overnight for approximately 12 h in the littoral (1–10 m depth), profundal (>20 m depth) and pelagic zones (0–6 m) in each lake. With an opened body cavity, the total number of *Diphyllobothrium* spp. cysts on the stomach, viscera and in the fish flesh was quickly visually assessed and recorded (CYST). In practice, this was done by counting the number of cysts from 0 to 50 and giving an estimate for intensities > 50. The stomach was emptied and then, together with other possible cyst-containing viscera and tissue, placed in digestive fluid (2% HCL with 5 gr/L pepsin and 9 gr/L NaCl) at room temperature to extract encysted plerocercoid larvae (Romeis 1968). This procedure also included fish that appeared to have no cysts and were assumed to be uninfected. After approximately 12 and 24 h, excysted plerocercoid larvae were collected and preserved in 4 % formaldehyde. They were later identified as *D. ditremum* or *D. dendriticum* following the morphological descriptions of Andersen, Ching & Vik (1987) and Andersen & Gibson (1989) using a disection scope with 40x to 400x magnification. In this way, a counted number of *Diphyllobothrium* spp. plerocercoid larvae recovered using the conventional digestive technique (LARV) was obtained.

Statistical analyses

Generalized linear modelling (GLM). A GLM was performed to analyze the overall association between CYST and LARV. The model also tested if the simplified methodology performed different on trout compared to charr. The GLM was based on Poisson distribution using $\log_{10}$CYST as explanatory variable, and $\log_{10}$LARV plus fish species (trout and charr) as response variables.

Linear modelling (LM). LMs were fitted to the fish species specific data, as the GLM indicated a difference between the two fish species. The two LMs used $\log_{10}$CYST as explanatory variable and $\log_{10}$LARV as response variable with an intercept adjusted to zero. The obtained log-transformed equations (based on log-transformed data) were back-transformed using the logarithm power rule ($\log (y) = m \times \log (x) = \log (x^m)$ which gives $y = x^m$) and used to acquire the calculated number of *Diphyllobothrium* spp. plerocercoid larvae (CALC) from CYST. Potential bias during back transformation of the log-transformed equations was ignored as the standard error of estimate of the regressions resulted in correction factors ≈ 1 for both fish species (Wood 1986).

The above models were validated by checking diagnostic plots. Only infected fish were used in the modelling approaches to avoid strong non-normality. Additional statistical analyses used the full dataset.

Correlation coefficients. To analyze the correlation between $\log_{10}$CYST and $\log_{10}$LARV, Pearson´s product-moment correlation coefficient was calculated for both fish species. As this test ignores potential skewness in the data, we supplemented with the non-parametric

Spearman´s rank correlation coefficient to test for correlations between the ranks of the two variables (Fowler, Cohen & Jarvis 1998).

Permutation testing: In order to evaluate the simplified method further, we analyzed if mean intensity, mean abundance and prevalence were significantly different whether based on CYST, CALC or LARV data. For this, we used the three lakes with the largest fish sample sizes among the sampled lakes (Lake Fjellfrøsvatn, Sagelvvatn and Takvatn). Due to skewed data, 10 000 cycled permutation testing was used (Greenacre & Primicerio 2014). In the case of mean intensity and mean abundance, three extreme cases were disregarded (two trout in Sagelvvatn and one trout in Takvatn) as they caused extreme bimodality in the permutated distribution.

Results

In total, 365 trout and 424 charr were sampled. Of these, 196 trout and 221 charr were used in the modelling approaches as they were infected with *Diphyllobothrium* spp.

According to the GLM, $\log_{10}$CYST was a significant explanatory variable for $\log_{10}$LARV ($p<0.001$). A near significant impact of fish species was also detected ($p=0.069$), indicating that the simplified methodology possibly works even better in charr compared to trout (see linear modelling results below).

Based on the Pearson´s product-moment correlation coefficient there was a strong correlation between $\log_{10}$CYST and $\log_{10}$LARV in both trout ($r_{(363)} = 0.85$, $p < 0.001$) and charr ($r_{(422)} = 0.90$, $p < 0.001$). The Spearman´s rank correlation coefficient also indicated a strong correlation in both trout ($r_s = 0.79$, $p < 0.001$) and charr ($r_s = 0.89$, $p < 0.001$).

Strong positive linear relationships between $\log_{10}$CYST and $\log_{10}$LARV were found in both trout ($y = 0.80x$, $r^2 = 0.90$, $F_{1,195} = 1689$, $p < 0.001$; Fig.1) and charr ($y = 0.94x$, $r^2 = 0.94$, $F_{1,220} = 3402$, $p < 0.001$; Fig. 1). For both fish species, almost all variation in $\log_{10}$LARV was explained by the variation in $\log_{10}$CYST. From the linear regressions, the following equations were established by back transformations using the logarithm power rule to estimate CALC for individual fish based on CYST:

Brown trout: $CALC = (CYST)^{0.80}$

Arctic charr: $CALC = (CYST)^{0.94}$

From the 365 trout caught, 109 were found to have no observable cysts. Of these, seven fish turned out to be infected (LARV: mean = 2, max = 6), giving 6 % false negatives. The number of trout recorded to have cysts were 256. In 60 of these, no larvae were retrieved (CYST: mean = 4, max = 30) giving 23 % false positives. From the 424 charr caught, 172 were recorded to have no observable cysts. Of these, 8 were infected (LARV: mean = 2, max = 3) giving 5 % false negatives. The number of charr estimated to have cysts were 252. In 31 of these, no larvae were retrieved (CYST: mean = 3, max = 30) giving 12 % false positives. False positives were not present when CYST > 30. However, when CYST > 100, considerable variation in the absolute difference between CYST and LARV occurred in both fish species

(Fig. 2). Most frequently, this was caused by higher CYST than LARV values, especially for trout (Fig. 1)

The practical functionality-test of the simplified methodology produced similar estimates of mean intensity and mean abundance of *Diphyllobothrium* spp. when based on CYST, and in particular the adjusted CALC data, compared to the LARV data (Table 2). This was generally true for both fish species and all three lakes (Lake Fjellfrøsvatn, Sagelvvatn and Takvatn) (Table 2). Concerning prevalence, there were also no significant differences between the calculations based on CYST and LARV data. This was true for both trout and charr in all three lakes. Except for trout in Sagelvvatn, the difference between the two estimations of prevalence was always below 10 percentage points (Table 2).

Discussion

Overall, the simplified methodology of using CYST as an indication of the total number of *Diphyllobothrium* spp. larvae in salmonids appeared as a functional alternative to the conventional and more laborious and time-consuming technique of excysting, collecting and counting all individual plerocercoid larvae. We found that $\log_{10}$CYST worked as a highly significant explanatory variable for $\log_{10}$LARV and a high species-specific correlation coefficient (Pearson´s product-moment) were also found between the two measures both for trout and charr. Additionally, almost all variation in $\log_{10}$LARV was explained by $\log_{10}$CYST in both salmonids. Despite providing a somewhat more coarse-grained estimate of the total number of *Diphyllobothrium* spp. larvae, we propose that this is a technique that is

well suited to be used in management surveys of salmonid fish populations for an assessment of the abundance of these parasites when time and/or resources are not available for more detailed parasitological studies. The correlation between CYST and LARV, plus the similarity between the CALC and LARV values, suggest that the cyst assessment methodology may also be applicable for certain scientific studies where a discrimination between the two *Diphyllobothrium* species is not required.

Our findings suggest that the simplified methodology might work slightly better for charr than for trout. This is possibly caused by the overall tendency of charr mostly to become infected with *D. ditremum* through heavy zooplankton feeding, whereas trout can have high intensities of *D. dendriticum* mainly aggregated through piscivory (Henriksen et al. 2016; Kuhn et al. 2016). The plerocercoid larvae of *D. dendriticum* are significantly larger than those of *D. ditremum* in the fish host, and hence have larger cysts that are also more irregular in shape. In the visual assessment, what potentially is thought to be multiple *D. ditremum* cysts might be a single *D. dendriticum* cyst, causing an overestimation of CYST, which may explain the lower fit of the simplified methodology in trout. Unfortunately, cyst counts does not work at the parasite species level, so any specific difference in the cyst estimation of the two parasite species cannot be explored.

In both fish species, considerable variation in the absolute difference between CYST and LARV was observed for CYST > 100, most commonly with the CYST-values being higher than the LARV-values. There are several potential explanations. For instance, at CYST > 100, the number of cysts becomes visually assessed rather than actually counted, and with high infection levels, the number of cysts may likely be overestimated. Furthermore, when

performing the digestion technique in the field, very heavily infected fish may also cause the sampler to compromise on parasite retrieval, as there usually is limited time to search for every single excysted larvae in the digestive fluid. Additionally, high parasite intensities may involve high intra- and interspecific competition between the parasites, potentially leading to enhanced mortality rates resulting in many empty cysts, which again will increase the mismatch between CYST and LARV. However, if the assessment of parasite abundance does not require a very precise count of the intensities in the most heavily infected individuals, the increased variation observed between CYST and LARV for the higher intensities, appears acceptable.

The occurrence of false negatives was low for both fish species, meaning that the CYST method successfully identifies uninfected individuals. The most plausible reason for the false negatives that did occur is that not all plerocercoids are encysted. Some might be in the process of migrating through the stomach wall, while others might be free (i.e. not encysted) in the viscera and muscle, especially in heavily parasitized individuals (Torres et al. 2010). Further, cysts mistaken as fat tissue or located in areas where they are hard to detect would also produce false negatives. False positives were more common but only occurred in fish with low infections. Several possible explanations may apply. One is that there is always some mortality among the plerocercoids within the cysts. Dead larvae will not withstand the digestive fluid, causing the CYST method to register an infection, whereas the LARV counts will not. False positives might also be due to difficulties in locating just one or a few excysted larvae in the murky digestive fluids, given the often limited time available for the search. Overall, however, the simplified methodology appeared sufficiently precise when it came to estimating the prevalence of *Diphyllobothrium* spp. in the salmonid community of a lake.

Contrary to estimating parasite abundance, the accuracy of the method in detecting infection versus no infection increased with increasing parasite intensity in the fish populations. The simplified methodology therefore seems highly useful in fish population management where obtaining the prevalence measure might be a quick way to assess the parasite burden of a host population. However, our study also reveals that reliable estimates of intensity and abundance are retrieved through this labor-efficient approach, providing data that are highly useful for management decisions. It should also be pointed out that a number of field workers with variable sampling experiences (including researchers, research technicians and students) have contributed in gathering the data used in this study, so a successful outcome of such studies does apparently not rely upon specific skills or extensive experience with this methodology.

For management purposes, a reduction of the parasite infection of a fish population may be highly desirable and beneficial. In the case of *Diphyllobothrium* spp., this genus has a global distribution and contains some of the most important fish-borne zoonoses among cestode parasites (Dick et al. 2001; Chair et al. 2005; Scholz et al. 2009). Transmission to humans might result in diphyllobothriasis, and reported cases of this condition are probably underdiagnosed (Kuchta et al. 2013). Traditions with eating raw fish in northern Europe, and the increasing popularity of sushi from salmonid species, certainly makes this a relevant subject concerning management of infected fish populations (Cabello 2007; Wicht et al. 2008; Jenkins et al. 2013). The *Diphyllobothrium* spp. plerocercoids, and particularly *D. dendriticum*, typically encyst in the muscle of the fish host at high intensities. It is especially at this site of infection that larvae are likely to be transmitted to humans where they have a prepatent period of a few weeks, but symptoms might stretch for significantly longer (Wicht

et al. 2008; Kuchta et al. 2013). As the simplified methodology presented in the current paper showed no false negatives at high parasite intensities, this suggests that the method is suitable when assessing the potential of a fish population as source of human infection and diphyllobothriasis. As cysts located in the muscle are detectable when dissecting the fish, the cyst assessment method might furthermore be a useful approach in respect to quantifying the amount of parasites located in fish muscle tissue.

High *Diphyllobothrium* spp. infections are unappetizing and leaves the fish unattractive and often unsuitable for human consumption. Additionally, *Diphyllobothrium* spp. can reach intensities that possibly also induce fish host mortality (Henricson 1977; Rahkonen et al. 1996; Hammar 2000). This is especially true for large and piscivorous individuals of trout and charr, which can be heavily infected (Kuhn et al. 2016; Henriksen et al. 2016; Siwertsson et al. 2016). Management efforts have been initiated to alleviate such parasite problems in fish populations, and have in cases such as e.g. reported by Amundsen & Kristoffersen (1990) and Klemetsen et al. (2002) been successful (see also Wood, Lafferty & Micheli 2010 for a review on the impact of fishing on rates of parasitism). However, the initiation of suitable management efforts requires good knowledge of the parasite infection of the fish population, and the simplified methodology to explore *Diphyllobothrium* spp. infections by assessing the number of cysts can in this respect be highly cost-efficient and useful.

In conclusion, our study reveals that an assessment of the number of *Diphyllobothrium* spp. cysts provides a reliable estimate of the total number of *Diphyllobothrium* spp. plerocercoid larvae in salmonids. Our findings thus suggest that this simplified methodology provides a sound proxy of the *Diphyllobothrium* spp. burden and have the potential to be used in e.g.

parasite assessment during fish management surveys, particularly if the time and resources for detailed parasite sampling and analysis are not available. An important downside of this simplified approach is the fact that it is not possible to discriminate between the two different *Diphyllobothrium* species known to be present in these salmonids (*D. ditremum* and *D. dendriticum*), but this may partly be compensated for by implementing the conventional methodology for a subsample of hosts. Nevertheless, for specific and detailed research objectives concerning the *Diphyllobothrium* species and their fish hosts, the conventional digestive technique is still to be preferred. For more simplified studies however, the cysts assessment method should be highly useful.

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References

Amundsen P.-A. & Kristoffersen R. (1990) Infection of whitefish (*Coregonus lavaretus* L. s.l.) by *Triaenophorus crassus* Forel (Cestoda: Pseudophyllidea): a case study in parasite control. *Canadian Journal of Zoology* **68**, 1187–1192.

Andersen K., Ching H.L. & Vik R. (1987) A review of freshwater species of *Diphyllobothrium* with redescriptions and the distribution of *D. dendriticum* (Nitzsch, 1824) and *D. ditremum* (Creplin, 1825) from North America. *Canadian Journal of Zoology* **65**, 2216–2228.

Andersen K.I. & Gibson D.I. (1989) A key to three species of larval *Diphyllobothrium* Cobbold, 1858 (Cestoda: Pseudophyllidea) occurring in European and North American freshwater fishes. *Systematic Parasitology* **13**, 3–9.

Bylund G. (1972) Pathogenic effects of a diphyllobothriid plerocercoid on its host fishes. *Commentationes Biologicae, Societas Scientiarum Fennica* **58**, 1–11.

Cabello F.C. (2007) Salmon aquaculture and transmission of the fish tapeworm. *Emerging Infectious Diseases* **13**, 169–171.

Chai J.-Y., Darwin Murrell K. & Lymbery A.J. (2005) Fish-borne parasitic zoonoses: status and issues. *International Journal for Parasitology* **35**, 1233–1254.

Curtis M.A. (1984) *Diphyllobothrium* spp. and the Arctic charr: parasite acquisition and its effects on a lake-resident population. In: *Biology of the Arctic charr: Proceedings of the International Symposium on Arctic Charr, Winnipeg, Manitoba, May 1981* (ed. by L. Johnson & B.L. Burns), pp. 395–411. University of Manitoba Press, Winnipeg.

Dick T.A. (2007) Diphyllobothriasis: the *Diphyllobothrium latum* human infection conundrum and reconciliation with a worldwide zoonosis. In: *Food-Borne Parasitic Zoonoses: Fish and Plant-Borne Parasites* (ed. by K.D. Murrell & B. Fried), pp. 151–184. Springer, London, United Kingdom.

Dick T.A., Nelson P.A. & Choudhury A. (2001) Diphyllobothriasis: update on human cases, foci, patterns and sources of human infections and future considerations. *The Southeast Asian Journal of Tropical Medicine and Public Health* **32,** 59–76.

Eloranta A., Knudsen R. & Amundsen P.-A. (2013) Niche segregation of coexisting Arctic charr (*Salvelinus alpinus*) and brown trout (*Salmo trutta*) constrains food web coupling in subarctic lakes. *Freshwater Biology* **58**, 207–221.

Esch G.W. (1994) Population biology of the diplostomatid trematode *Uvulifer ambloplitis*. In: Parasitic and Infectious Diseases: Epidemiology and Ecology (ed. by M.E. Scott & G. Smith), pp. 321–335. Academic Press, San Diego.

Fowler J., Cohen L. & Jarvis P. (1998) *Practical Statistics for Field Biology*. Second Edition. John Wiley & Sons Ltd., England.

Greenacre M. & Primicerio R. (2014) *Multivariate Analysis of Ecological Data*. Fundacion BBVA, Bilbao.

Halvorsen O. (1970) Studies of the helminth fauna of Norway XV: on the taxonomy and biology of plerocercoids of *Diphyllobothrium* Cobbold, 1858 (Cestoda, Pseudophyllidea) from north-western Europe. *Nytt Magasin for Zoologi* **18**, 113–174.

Halvorsen O. & Andersen K. (1984). The ecological interaction between arctic charr, *Salvelinus alpinus* (L.), and the plerocercoid stage of *Diphyllobothrium ditremum*. *Journal of Fish Biology* **25**, 305–316.

Hammar J. (2000) Cannibals and parasites: conflicting regulators of bimodality in high latitude Arctic char, *Salvelinus alpinus*. *Oikos* **88**, 33–47.

Henricson J. (1977) The abundance and distribution of *Diphyllobothrium dendriticum* (Nitzsch) and *D. ditremum* (Creplin) in the char *Salvelinus alpinus* (L.) in Sweden. *Journal of Fish Biology* **11**, 231–248.

Henriksen E.H., Knudsen R., Kristoffersen R., Kuris A.M., Lafferty K.D., Siwertsson A. & Amundsen P.-A. (2016) Ontogenetic dynamics of infection with *Diphyllobothrium* spp. cestodes in sympatric Arctic charr *Salvelinus alpinus* (L.) and brown trout *Salmo trutta* L. *Hydrobiologia* DOI: 10.1007/s10750-015-2589-2.

Jenkins E.J., Castrodale L.J., de Rosemond S.J.C., Dixon B.R., Elmore S.A., Gesy K.M., Hoberg E.P., Polley L., Schurer J.M., Simard M. & Thompson R.C.A. (2013) Tradition and transition: parasitic zoonoses of people and animals in Alaska, northern Canada, and Greenland. In: *Advances in Parasitology* (ed. by D. Rollinson), pp. 33–204. Elsevier Ltd., Academic Press.

Klemetsen A., Amundsen P.-A., Grotnes P.E., Knudsen R., Kristoffersen R. & Svenning M.-A. (2002) Takvatn through 20 years: long-term effects of an experimental mass removal of Arctic Charr, *Salvelinus alpinus*, from a subarctic lake. *Environmental Biology of Fishes* **64**, 39–47.

Knudsen R., Amundsen P.-A. & Klemetsen A. (2002) Parasite-induced host mortality: indirect evidence from a long-term study. *Environmental Biology of Fishes* **64**, 257–265.

Knudsen R., Amundsen P.-A., Nilsen R., Kristoffersen R. & Klemetsen A. (2008) Food borne parasites as indicators of trophic segregation between Arctic charr and brown trout. *Environmental Biology of Fishes* **83**, 107–116.

Knudsen R. & Klemetsen A. (1994) Infections of *Diphyllobothrium dendriticum*, *D. ditremum* (Cestoda), and *Cystidicola farionis* (Nematoda) in a north Norwegian population of Arctic charr (*Salvelinus alpinus*) during winter. *Canadian Journal of Zoology* **72**, 1922–1930.

Kuhn J.A., Frainer A., Knudsen R., Kristoffersen R. & Amundsen P.-A. (2016) Effects of fish species composition on *Diphyllobothrium* spp. infections in brown trout – is three-spined stickleback a key species? *Journal of Fish Diseases* DOI: 10.1111/jfd.12467.

Kuhn J.A., Kristoffersen R., Knudsen R., Jakobsen J., Marcogliese D.J., Locke S.A., Primicerio R. & Amundsen P.-A. (2015) Parasite communities of two three-spined stickleback populations in subarctic Norway—effects of a small spatial-scale host introduction. *Parasitology Research* **114**, 1327–1339.

Kuchta R., Brabec J., Kubáčová P. & Scholz T. (2013) Tapeworm *Diphyllobothrium dendriticum* (Cestoda) - neglected or emerging human parasite? *PLoS Neglected Tropical Diseases* **7**, 1–8.

Marcogliese D.J. (2004) Parasites: small players with crucial roles in the ecological theater. *EcoHealth* **1**, 151–164.

Meyer M.C. & Vik R. (1961) *Sparganum sebago*, incidence and location in host fishes. *The Journal of Parasitology* **47**, 56.

Rahkonen R., Aalto J., Koski P., Särkkä J. & Juntunen K. (1996) Cestode larvae *Diphyllobothrium dentriticum* as a cause of heart disease leading to mortality in hatchery-reared sea trout and brown trout. *Diseases of Aquatic Organisms* **25**, 15–22.

Rahkonen R. & Koski P. (1997) Occurrence of cestode larvae in brown trout after stocking in a large regulated lake in northern Finland. *Diseases of Aquatic Organisms* **31**, 55–63.

Romeis B. (1968) *Mikroskopische Technik*. R. Oldenbourg Verlag, München.

Sánchez-Hernández J. & Amundsen P.-A. (2015) Trophic ecology of brown trout (*Salmo trutta* L.) in subarctic lakes. *Ecology of Freshwater Fish* **24**, 148–161.

Scholz T., Garcia H.H., Kuchta R. & Wicht B. (2009) Update on the human broad tapeworm (genus *Diphyllobothrium*), including clinical relevance. *Clinical Microbiology Reviews* **22**, 146–160.

Siwertsson A., Refsnes B., Frainer A., Amundsen P.-A. & Knudsen R. (2016) Divergence and parallelism of parasite infections in Arctic charr morphs from deep and shallow lake habitats. *Hydrobiologia* DOI: 10.1007/s10750-015-2563-z.

Torres P., Leyán V. & Puga S. (2012) Prevalence, intensity, and abundance of infection and pathogenesis caused by diphyllobothriosis in vulnerable, native fish and introduced trout in Lake Panguipulli, Chile. *Journal of wildlife diseases* **48**, 937–950.

Torres P., Lopez J.C., Cubillos V., Lobos C. & Silva R. (2002) Visceral diphyllobothriosis in a cultered rainbow trout, *Oncorhynchus mykiss* (Walbaum), in Chile. *Journal of Fish Diseases* **25**, 375–379.

Torres P., Quintanilla J.C., Rozas M., Miranda P., Ibarra R., San Martín M.F., Raddatz B., Wolter M., Villegas A., Canobra C., Hausdorf M. & Silva R. (2010) Endohelminth parasites from salmonids in intensive culture from southern Chile. *Journal of Parasitology* **96**, 669–670.

Vik R. (1964) The genus *Diphyllobothrium*: an example of the interdependence of systematics and experimental biology. *Experimental Parasitology* **15**, 361–380.

Wicht B., de Marval F., Gottstein B. & Peduzzi R. (2008) Imported diphyllobothriasis in Switzerland: molecular evidence of *Diphyllobothrium dendriticum* (Nitsch, 1824). *Parasitology Research* **102**, 201–204.

Wood A. (1986) A potential bias in log-transformed allometric equations. Wader Study Group Bulletin 47, 17–19.

Wood C.L., Lafferty K.D. & Micheli F. (2010) Fishing out marine parasites? Impacts of fishing on rates of parasitism in the ocean. *Ecology Letters* **13**, 761–775.

Table 1 Physical parameters and catch data from the nine lakes sampled in northern Norway.

							Brown trout			Arctic charr		
Lake	Latitude (North)	Longitude (East)	Surface area (km^2)	Altitude (m.a.s.l.)	Max. depth (m)	Sampling time (dd.mm.yy)	Sample size	Fish length mean (mm)	Fish length range (min-max)	Sample size	Fish length mean (mm)	Fish length range (min-max)
Fjellfrøsvatn	69°05'	19°20'	6.5	125	88	13.-14.08.10	29	229	114-545	73	171	78-412
Fjerdevatn	67°46'	15°58'	2.3	79	35	07.08.13	37	265	135-447	0	-	-
Forsanvatn	67°54'	15°42'	4.8	257		03.08.13	33	275	125-356	0	-	-
Makkvatn	67°50'	15°49'	3.0	123		05.08.13	60	221	138-310	17	230	162-252
Rekvatn	67°56'	16°04'	7.4	297		05.-06.08.13	18	183	124-310	18	182	108-270
Sagelvvatn	69°11'	19°06'	5.1	91	80	10.-12.08.10	95	215	85-543	95	202	83-340
Skilvatn	68°04'	15°53'	3.3	35	53	02.-04.08.13	7	245	175-303	95	203	133-328
Storvatn	67°56'	16°00'	2.6	157	48	31.07.13	7	249	167-365	0	-	-
Takvatn	69°07'	19°05'	15	214	80	11.-13.08.15	79	215	83-711	126	205	80-405

Table 2 Mean intensity, mean abundance and prevalence of *Diphyllobothrium* spp. infecting brown trout and Arctic charr in three Norwegian lakes, based on either CYST, CALC or LARV. (CYST) assessed number of *Diphyllobothrium* spp. cysts. (CALC) calculated number of *Diphyllobothrium* spp. plerocercoid larvae (brown trout: CALC = $(CYST)^{0.80}$, Arctic charr: CALC = $(CYST)^{0.94}$). (LARV) counted number of *Diphyllobothrium* spp. plerocercoid larvae recovered using a conventional digestive technique.

	Brown trout			Arctic charr		
	CYST	CALC	LARV	CYST	CALC	LARV
Mean intensity						
Fjellfrøsvatn	57.7	23.8	20.4	19.4	15.9	11.2
Sagelvvatn	[a]10.5*	[b]6.0	[c]4.2*	22.1	17.9	16.9
Takvatn	[d]10.5	[e]5.7	[f]9.5	8.1	8.5	6.9
Mean abundance						
Fjellfrøsvatn	11.9	4.9	4.9	2.1	1.7	2.0
Sagelvvatn	[g]4.7**	[h]2.7*	[i]1.2*(**)	13.7	11.1	10.5
Takvatn	[j]5.8	[k]3.2	[l]4.6	3.5	3.0	2.9
Prevalence						
Fjellfrøsvatn	20.7	--	24.1	11.0	--	17.8
Sagelvvatn	46.3	--	29.5	62.1	--	62.1
Takvatn	55.7	--	49.4	43.7	--	34.1

Permutation test between CYST or CALC and LARV: *$p < 0.05$,** $p < 0.001$, [no mark] $p > 0.05$
Including omitted fish: mean intensity = [a]78.2, [b]21.3, [c]19.1, [d]55.7, [e]15.5, [f]42.1; mean abundance = [g]36.2, [h]9.9, [i]5.6, [j]31.0, [k]8.7, [l]20.8

Legends

Figure 1 Linear regression between assessed number of *Diphyllobothrium* spp. cysts (CYST) and counted number of *Diphyllobothrium* spp. plerocercoid larvae recovered using a conventional digestive technique (LARV), for brown trout (a) and Arctic charr (b). Solid lines indicates fitted linear models. Dashed lines indicate identity lines 1:1. The points light grey—black tone denotes frequency of observation. Fish with zero values for CYST and/or LARV are included in the plot. Note that all axes are logarithmic.

Figure 2 Variation in absolute difference between assessed number of *Diphyllobothrium* spp. cysts (CYST) and counted number of *Diphyllobothrium* spp. plerocercoid larvae recovered using a conventional digestive technique (LARV), for brown trout (a) and Arctic charr (b). Boxplots show median (bold lines), upper, and lower quartiles (top and bottom borders of the boxes), minimum and maximum (top and bottom endings of the vertical lines) as well as outliers (black dots). N of each group is given in the top. Note the different scales in the axes.

Fig 1

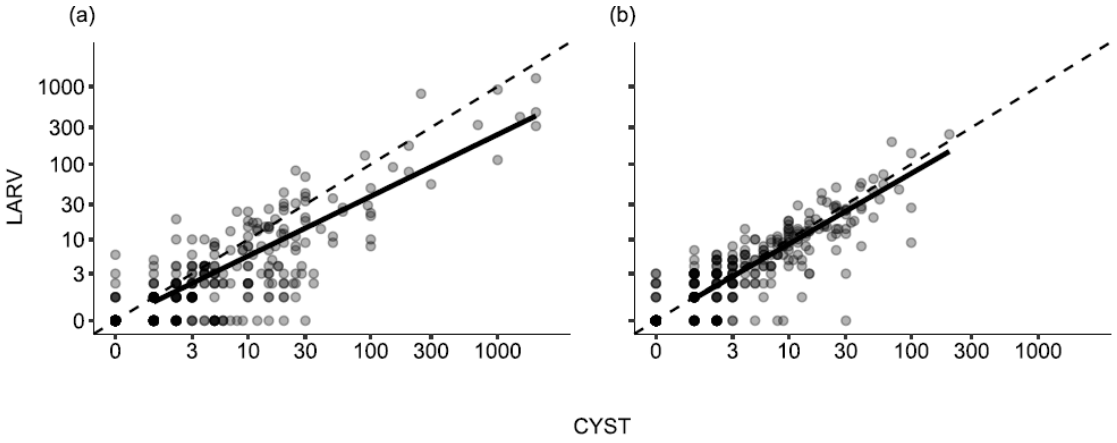
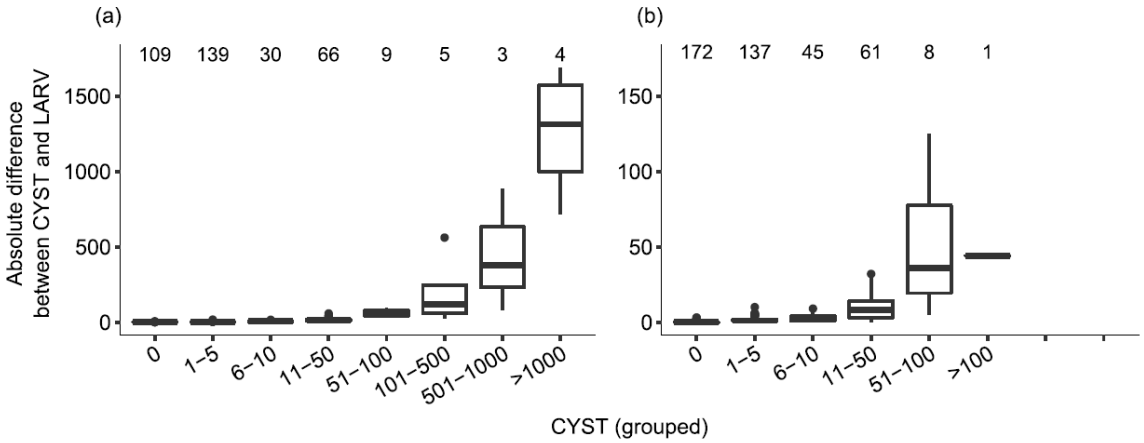


Fig 2


(a)
(b)
109 139 30 66 9 5 3 4
172 137 45 61 8 1
Absolute difference between CYST and LARV
1500
1000
500
0
150
100
50
0
0
1–5
6–10
11–50
51–100
101–500
501–1000
>1000
>100
CYST (grouped)