Review

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Drug resistance in sea lice: a threat to salmonid aquaculture

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Sea lice are copepod ectoparasites with vast reproductive potential and affect a wide variety of fish species. The number of parasites causing morbidity is proportional to fish size. Natural low host density restricts massive parasite dispersal. However, expanded salmon farming has shifted the conditions in favor of the parasite. Salmon farms are often situated near wild salmonid migrating routes, with smolts being particularly vulnerable to sea lice infestation. In order to protect both farmed and wild salmonids passing or residing in the proximity of the farms, several measures are taken. Medicinal treatment of farmed fish has been the most predictable and efficacious, leading to extensive use of the available compounds. This has resulted in drug-resistant parasites occurring on farmed and possibly wild salmonids.

Massive technological progress above and below sea level

Terrestrial food production represents the main protein source in the industrialized world. Due to a rapidly growing human population, and thus a deficiency of traditional protein resources, alternative ways to provide for the increase in nutrient demand are being sought. In 2006, the world aquaculture production of fish contributed to 47% of the world's food fish supply, mainly carps and other cyprinids (59%) but also salmonids. Approximately 7% of the world's fish production in aquaculture comes from salmonid farming [1]. Fish farming in sea water possesses vast potential for protein processing [2], with the most heavily industrialized production being farming of Atlantic salmon. Developing from small-scale production to a massive industry within 40 years, the optimal techniques for fish farming are probably yet to be identified. Several pathogens are compromising salmon production, most of which are being addressed through vaccines and other precautions. However, caligid copepods have proved to be a major constraint to biological sustainability. In 2002, Denholm *et al*. [3] reviewed the development of sea lice resistance towards available compounds. Intensive research has been conducted with several sea lice species since then, catapulting the knowledge about parasite–fish interactions and molecular mechanisms to the next level.

A state of constant increase

Commercial farming of salmonids was initiated in mid-Norway in the late 1960s (see Store Norske leksikon

Glossary

Acetylcholine (ACh): a neurotransmitter present in cholinergic synapses.
Acetylcholine esterase (AChE): an enzyme involved in inactivation of acetylcholine.
Azamethiphos (AZA): an organophosphate targeting acetylcholine esterase, used as a delousing compound applied as bath treatment, and has been used since ~1994.
Benzoyl ureas: a class of chemical compounds that inhibit molting in several parasite or pest species. These compounds inhibit the synthesis of chitin.
Cypermethrin (CYPER): a compound within the class of pyrethroids; it is derived from the organic compound pyrethrin.
Deltamethrin (DELTA): a compound from the group of pyrethroids. It is structurally similar to cypermethrin.
Diflubenzuron (DIFLU): a compound from the group of benzoyl ureas.
Emamectin benzoate (EMB): a chemical compound within the class of avermectins. This compound acts mainly as activator of chloride channels in membranes.
Fitness cost: the loss of a feature or a favorable metabolic pathway through changes in genetic material to avoid the effects of xenobiotics.
Hydrogen peroxide (H_2O_2): a disinfectant with insecticidal and ovicidal properties.
γ-Aminobutyric acid (GABA): a neurotransmitter present in both vertebrates and invertebrates.
Glutamate (GLU): a proteinogenic amino acid and neurotransmitter.
Glutathione S-transferase (GST): a class of isoenzymes with a range of functions, including metabolism of xenobiotics.
Integrated pest management (IPM): a theory for extirpation of pest organisms through a combination of complex measures.
Mixed function oxidases (CYP): metabolic enzymes of the cytochrome P450 class.
Organophosphates (OPs): a class of chemical compounds that have been utilized as insecticides in agriculture for more than 50 years. These compounds inhibit acetylcholine esterase, leading to paralysis.
P-glycoprotein (P-gp): a protein of the cell membrane responsible for the efflux of a range of substances from the cell. P-gp is also known as multidrug resistance protein 1.
Pyrethroids (PYRs): a class of compounds that includes the substances cypermethrin and deltamethrin.
Refugia: parasites not treated with medicinal compounds to let them spread their genetic material to the population of reduced sensitivity. In theory, refugia will slow down resistance development.
Teflubenzuron (TEFLU): a compound from the group of benzoyl urea compounds.
Therapeutic index: a mathematic factor describing the medicinal dose toxic to salmon/dose used to remove sea lice off fish.
Voltage-dependent sodium channels (VDSC): channels specific to sodium ions, opened by depolarisation of the membrane.
Xenobiotics: foreign chemical substances present in living organisms.

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(https://snl.no/fiskeoppdrett)) with the seawater cultivation phase conducted in floating net pens, allowing for the exchange of water and its contents with the environment. Fish farms are considered a major factor for spreading sea lice to wild salmonids in Europe and North America [4]. When the critical parasite abundance level is exceeded, the host could eventually suffer from osmotic stress and secondary infections, leading to mortalities [5]. The interplay of farmed salmonids, parasites, and wild salmonids is, however, a complex matter. Species-dependent ability to reject the parasite, interaction between the parasite and the host immune system, self-imposed delousing in rivers, and fish size are all important factors determining the outcome for the host (reviewed by Torrisen *et al.* [6]). Thus, sea lice have varying impacts on wild salmonid populations [6,7].

Sea lice levels in salmon farms are under comprehensive surveillance throughout the sea water production period. Medicinal treatments have historically been the most predictable measures to prevent the occurrence of high sea lice abundance. The possibility of infecting vulnerable wild salmonids residing in the proximity of fish farms is a major concern behind the monitoring of sea lice levels. Altogether, sea lice parasites constitute a serious threat to sustainable salmon farming in the main producing countries, Canada, Chile, the Faroe Islands, Ireland, Norway, and Scotland, as the total production of Atlantic salmon has almost doubled from 1.1 billion tons in 2002 to 2.1 billion tons in 2012 (FAO Cultured Aquatic Species Information Programme Salmo salar (http://www.fao.org/fishery/culturedspecies/Salmo_salar/en)). Spillover from medicinal treatments to remove sea lice is potentially harmful to other organisms such as lobsters and shrimps [8]. Extensive use of medicines has resulted in an inevitable drift towards resistance. This imposes a huge threat for fish health and welfare, the environment, the economy in salmonid production, and for seafood production in general. In summary, these parasites represent a massive economical and biological obstacle to fish farming companies in all salmon-producing countries.

Life cycle and current combat strategies

In Europe and Canada, the most frequently occurring sea louse is *Lepeophtheirus salmonis* (Krøyer), whereas *Caligus rogercresseyi* (Boxshall and Bravo) is its counterpart in the Southern Hemisphere. A third species, *Caligus elongatus* (Nordmann), is to some degree prevalent in Europe and Canada, affecting a variety of salmonid and non-salmonid species. *C. elongatus* is only sporadically subject to research because of its low prevalence and limited influence on fish morbidity. All three copepods are crustaceans, and their life cycle consists of eight stages; three of these stages are planktonic whereby the third stage (copepodid) infects the fish, and five of these stages are parasitic instars. Regarding *L. salmonis*, two instars (chalimus I and II) attach to the fish by a protein filament, whereas the following three (pre-adult I, II, and adult) are able to move on the host while grazing on mucus and blood. The *Caligus* species also comprise five parasitic instars: the first four (chalimus I–IV) attach to the host by a protein filament, with the latter molting to the adult stage [9]. The developmental time from the extrusion of egg strings to adults is temperature dependent. For *L. salmonis*, it is approximately 40 days for males and 50 days for females at 10°C, but shorter for *C. rogercresseyi* (reviewed by Costello [10]).

Apart from chemical intervention through bath treatments and medicated feed, several non-chemical methods are utilized to remove or reduce the number of parasites attaching to farmed fish. Fallowing, synchronized treatments within geographic zones, cleaner fish, delousing laser, and plankton shielding skirts are already in use, with others such as snorkel cages and enclosed cages on the verge of commercial introduction. Despite the use of alternatives to chemical treatment, extensive use of medicinal compounds combined with limited access to effective chemical compounds has led to widespread resistance towards the most applied medicinal products. The evolving reduced sensitivity in sea lice is a good example of microevolution, and shows how humans can influence nature considerably in a relatively short time frame.

Addressing a prevailing parasite in fish farming, this text will review current knowledge of resistance against chemical treatment agents in *L. salmonis* and *C. rogercresseyi*. The emergence of more accessible molecular methods has already contributed to a rapid increase in knowledge regarding this issue.

General definitions of resistance

The World Health Organization expert committee in 1957 defined resistance to insecticides as the 'development of an ability, in a strain of insects, to tolerate doses of toxicants which would prove lethal to the majority of individuals in a normal population of the same species' [11]. The degree of reduced susceptibility needed to survive an antiparasitic treatment differs between agricultural pests and parasites attached to a vertebrate host. Successful treatments of agricultural pests are mainly dependent on the dose, whereas for parasitic pests, the maximum applicable amount is determined by the host's ability to withstand the toxicity as well as the parasite's susceptibility to the compound. For several anti-sea lice medicines the therapeutic index (see Glossary) is small; thus, only minor reductions in sea lice susceptibility may present as clinical resistance in the field [12–14]. Resistance development is driven by the survival and reproduction of the less sensitive individuals [15].

The expression "resistant sea lice" is mainly applied for populations showing a high degree of reduced sensitivity in bioassays. A therapeutic failure in the field could be caused by a reduced sensitivity to the applied medicinal compound, but also by suboptimal treatment regimens, such as insufficient drug dispersal, poor medicine management, or badly adjusted feeding procedures.

Several genetic resistance mechanisms have been identified in arthropods. These include point mutations in the chemical's target gene rendering the protein insensitive to the chemical, upregulation of genes for detoxifying metabolism of the chemical by the parasite, and upregulation of efflux pumps in the parasite's intestine leading to decreased uptake of chemicals given to the fish as medicated feed. Similarly, changes in genes coding for cuticle thickness or

Table 1. A systemic approach to medicinal compounds, their mode of action, and resistance mechanisms in relation to sea lice treatment[a]

Substance	Mode(s) of action	Resistance mechanisms	Resistance in sea lice	Refs
Emamectin benzoate. Oral treatment	Activation of glutamate- and GABA-gated chloride channels, thereby reducing the cell's excitability. Mechanisms probably not fully elucidated	Resistance in nematodes and arthropods. Overexpression of P-gps, CYPs, GST, carboxyl esterases. Changes in glutamate- and histidine-gated ion channels	Widespread. Mechanisms unclear. Overexpression of metabolic enzymes, P-gps, and downregulation of GABA-gated chloride channels and neuronal AChE receptors	[55–62]
Benzoyl ureas, diflubenzuron and teflubenzuron. Oral treatment	Inhibition of chitin synthesis, rendering the parasites unable to detach from their exuviae during molting	Resistance in arthropods. Overexpression of CYPs. Mutation in the chitin synthase gene	Not reported	[63–65]
Hydrogen peroxide. Bath treatment	Not fully elucidated. Etching action. Gas bubbles in the body rendering the parasites unable to hold on to a surface. Also used against amoebic gill disease	Resistance in bacteria and fungi. Overexpression of metabolic enzymes such as catalase, glutathione peroxidase, GST in mammalian cells, bacteria, and fungi	Reported from Norway and Scotland. Mechanism not elucidated	[66–72]
Azamethiphos. Bath treatment	Inhibition of AChEs in cholinergic synapses, leading to excitation and subsequently paralysis	Resistance in arthropods. Mutations in genes coding for AChE. Overexpression of metabolic enzymes. Described already in the 1960s	Reported from the North Atlantic Ocean. Mutation in one of the genes coding for AChE	[3,73,74]
Pyrethroids, deltamethrin and cypermethrin. Bath treatment	Disruption of neuronal signals through interference with VDSCs, leading to excitation and subsequently paralysis	Resistance in arthropods. Also reported in non-target aquatic species. Overexpression of metabolic enzymes such as CYPs, GST, superoxide dismutase, mutations in the *VDSC* gene, and reduced cuticle penetration	Widespread. Metabolic resistance most likely	[75–84]

[a]Abbreviations: AChE, acetylcholine esterase; CYPs, mixed function oxidases; GABA, γ-aminobutyric acid; GST, glutathione S-transferase; P-gps, P-glycoproteins; VDSC, voltage-dependent sodium channel.

other protective mechanisms that decrease the penetration of the chemical into the parasite can be a resistance mechanism [16].

Substances from five groups of medicinal compounds are used to combat sea lice: avermectins, benzoyl ureas, disinfectants, organophosphates (OPs), and pyrethroids (PYRs). Their use in different countries, modes of action, and resistance mechanisms are summarized in Tables 1 and 2. Selection of highly resistant individuals may be facilitated by the use of off-label medicines, when inadequate treatment regimens are upheld, or when drugs with long half-lives are utilized [17]. It may be argued that national imposed treatment thresholds are an important factor for resistance selection. By intending to reduce the

Table 2. A historical presentation of medicinal compounds; where and when they have been used to treat fish for sea lice infestations

Compound	Canada	Chile	Faroe Islands[a]	Ireland	Norway	Scotland	Refs
Organophosphates	Between mid-1990s to early 2000. Now sporadically, emergency drug release	Metriphonate 1981–1985. Dichlorvos 1985–2000. AZA since 2013	Since 1997	Yes	Metriphonate 1974–1996. Dichlorvos 1986–1997. AZA 1994–1999 and since 2008[b]	Dichlorvos 1979–1997. AZA since 1994	[6,43,85–88]
Pyrethroids	Emergency drug release 2009–2010	Since 2007	Since 1997	DELTA since 2006[c]	Since 1994	CYPER since 1997, DELTA since 2008[c]	[35,85,88,89]
Emamectin benzoate	Since 1999	Since 1999. Only available medicine between 2000 and 2007	Since 1999	Yes	Since 1998	Since 2000	[33,86,87,90,91]
Benzoyl ureas	No	Since 2009	Since 1997	Yes	Since 1996[b]	Since 2007	[43,86,88,90]
Hydrogen peroxide	Sporadically, emergency drug release	Since 2007	Since 2011	No marketing authorization. Sporadic use	1993–1997. Reintroduced 2009[b]	1993–1999. Reintroduced in 2009	[12,43,87,88,92,93]

[a]Data provided by Mørkøre, B., Food and Veterinary Authority, Faroe Islands, personal communication.

[b]Data from the Norwegian Institute of Public Health (http://www.fhi.no/artikler/?id=109416).

[c]Data provided by Martinsen, B., Pharmaq Norway, personal communication.

number of infective copepodids, the treatment threshold for *L. salmonis* is in some areas in the region of 0.5–1 parasite per fish. This can result in numerous treatments during the production cycle, thereby inducing a highly effective process of selecting resistant individuals. Resistance to more than one xenobiotic occurring in individuals or a population is called cross resistance or side resistance when the underlying mechanism is identical. When several mechanisms are simultaneously carried by a parasite, this is called multiple resistance. There is, however, some confusion regarding these expressions, as there is no current standardized definition [18].

The fitness cost of resistance mechanisms in sea lice are to some degree described. Espedal *et al*. [19] found no fitness cost in generation F4 in a laboratory-reared lice strain resistant to emamectin benzoate (EMB). Bravo *et al*. [20] found no increase in sensitivity of *C. rogercresseyi* after 11 passages of an EMB-resistant strain in the laboratory. This applies also for OPs and PYRs. In our laboratory, OP- and PYR-resistant strains of *L. salmonis* have been reared for more than ten generations without loss of the resistance trait (Aaen *et al*., unpublished). Even though resistant traits against the most commonly used medicinal products in salmon lice may be associated with some fitness costs, these do not seem to be substantial for any genotype.

Refugia: isolated populations

Occurrence of refugia is important in resistance development, as untreated parasites may possess wild-type genes, and thus slow down the drift towards highly resistant populations. In the present salmonid farming areas, refugia will exist either on wild hosts or on hosts in parts of farms not treated. The latter is not commonly seen in fish farming compared to agriculture. This leaves wild salmonid fish as identified refugial hosts for *L. salmonis* in Europe, whereas in Western Canada, the three-spine stickleback is also reported to carry the parasite [21]. In Chile, the most common native host for *C. rogercresseyi* is thought to be *Eleginops maclovinus*, supplying refugia year-round, probably participating to slow down the development of resistance [22].

Resistance development: geographical overview

Information about the severity of reduced sensitivity in sea lice populations from salmon-producing countries is scarce. Only Norway has implemented a nationwide surveillance program for resistance monitoring based on bioassay data. In other countries, there are some organized efforts. Some of the generated data are published through various channels, whereas some are kept within the industry and research groups.

Norway. A number of incidents of non-effective treatments with EMB and PYRs have been reported since 2008. Since 2009, reports of treatment failures with azamethiphos (AZA) exist. The reports validate the significant increase in the relative use of delousing agents in Norway [23]. However, no official monitoring to determine the extent of the problem was in place before the surveillance program initiated by the Norwegian Food Safety Authority was conducted in 2013. The basis for the survey was a simplified bioassay method [24], demonstrating that reduced sensitivity towards AZA, deltamethrin (DELTA), and EMB was present in all counties except Finnmark, the northernmost county in Norway [25]. The survey also revealed that the areas with the most severe problems were located in the Hordaland county in the south west, the Nord-Trøndelag county in mid-Norway, and the northern part of the adjacent county Nordland. In these areas, resistance towards all agents in the program (AZA, DELTA, and EMB) was widespread, whereas in many cases the same population displayed resistance traits towards all the above-mentioned compounds simultaneously, a scenario of multiple resistance. In the other areas, the situation was more subtle, with variations from susceptible parasites to resistance towards one or several agents. Resistance towards hydrogen peroxide (H_2O_2), a compound also used against amoebic gill disease [26], has also been reported from mid-Norway [72]. For the chitin synthesis inhibitors diflubenzuron (DIFLU) and teflubenzuron (TEFLU), no evidence for resistance is reported, although some reduced treatment efficacy was seen in 2011 and 2012 [25].

Scotland. Failing efficacy of EMB has been reported since 2008. Heumann *et al*. [27] reported a sevenfold reduced sensitivity towards EMB in a salmon lice population isolated from the west coast in 2008. In an epidemiological study, Lees *et al*. [28] demonstrated a gradual decline in efficacy of this compound since 2004. However, no recent sensitivity data have been published for AZA, PYRs, or H_2O_2.

Ireland. There are no recently published reports of sensitivity issues in sea lice from this country. Jackson [29] indicated that reduced treatment efficacy, herein possibly reduced sensitivity, could be a contributing factor to an observed rise in the average number of parasites per fish in the period 2005–2007.

Canada. Several publications describe surveillance projects for sea lice sensitivity towards chemotherapeutants. In a survey conducted on the west coast of Canada in 2010–2012, only 1 out of 22 bioassays clearly indicated development of tolerance towards EMB, the only applied treatment in the region [30]. Analysis of treatment efficacy data also indicated that reduced sensitivity towards EMB did not appear to be a problem in British Columbia. On the east coast, the scenario is different, emphasized by the fact that Pacific and Atlantic *L. salmonis* are different species [31]. Furthermore, the presence of alternate hosts is vast on the Pacific side, and almost negligible on the Atlantic side. Reduced efficacy of EMB became a concern in the Bay of Fundy in 2008 [32]. However, a statistical analysis of EMB treatment efficacy data between 2004 and 2008 revealed that the efficacy of this compound declined gradually during these years [33,34]. Information about other treatments is scarce. DELTA was used in the region in 2009 and 2010. Analysis of treatment efficacy data demonstrated consistently suboptimal efficacy on adult female parasites (< 70% clearance) and bioassays revealed sensitivity levels elevating tenfold between parasite populations [35]. PYR had not been used in the region prior to the survey. The results are in retrospect explained by methodological errors [36]. In Canada, AZA, TEFLU, and H_2O_2 have

also been used to a limited extent over the last 5 years [32], but there are no reports of reduced sensitivity.

Chile. Reduced sensitivity has been reported towards EMB and DELTA in the sea lice species causing the most problems in Chilean salmon aquaculture, *C. rogercresseyi*. Bravo *et al.* [37] reported that treatment failures with EMB had occurred since 2006, and bioassays demonstrated sensitivity levels that corresponded with the resistant *L. salmonis*. Helgesen *et al.* [23] found that in 2012, the sensitivity of *C. rogercresseyi* had decreased by a factor of 5–10 since the introduction of DELTA as a delousing agent in Chile in 2008. Information about the sensitivity status for the other available treatments, AZA, DIFLU, and H_2O_2, is unavailable.

In conclusion, resistance towards available treatments is rapidly emerging in all major salmon-producing countries. This is supported by the findings from Besnier *et al.* [38]. Using a novel diagnostic tool, the SNP-chip, a genome sequence connected to EMB resistance found in sea lice from all countries examined [Canada, the Faroe Islands, Ireland, Norway, and the UK (Shetland)] was reported. Although there is limited information about the scale of the problem in Scotland and Ireland, there is reason to believe, based on the reports noted above, that resistance causes serious problems in all salmonid farming regions except Western Canada.

Monitoring the sensitivity level of sea lice

Traditional resistance detection: bioassays

Bioassays (biological assays) are experiments in which a living organism, tissues, or cells are used as a test subject [39]. Regarding sea lice, the expression is commonly used for quantitative response bioassays using a binary model for recording response, that is, dead versus alive, and one explanatory variable, the differing quantities of a treatment chemical. To assess sensitivity levels in *L. salmonis*, bioassays for PYR, EMB, and AZA have been developed [24,40–42], and for EMB and DELTA for *C. rogercresseyi* [24,37]. These original bioassay protocols are relatively comprehensive with regard to the number of parasites, equipment, and labor needed to perform the assays. Subsequently simpler bioassay protocols have therefore been developed [24,44]. Several biases are connected to sensitivity testing by bioassays, such as equipment setup and the personnel performing the tests. The final outcome is eventually connected to sea lice behavior, as dead versus alive is measured by parasite mobility. This contributes to low test sensitivity. Bioassays therefore have limitations when comparing resistance levels between different regions, and also as a tool to predict treatment efficacies as shown for EMB [42]. Although bioassays are valuable tools in resistance detection when the resistance mechanisms are unknown, the need for more accurate and simpler methods has led to extensive research to identify biomarkers and to develop molecular test methods.

Novel methods: molecular sequencing

Specific resistance mechanisms can in some cases be identified through phenotypic traits displayed by the resistant organism (Figure 1). Molecular methods can be developed for known mechanisms. They do not require that the parasites are alive when enrolled and can be set up as automated assays with high precision, high-throughput potential, and a reduced total of costs. Candidate gene screening is, however, labor intensive and may require substantial work before a suitable biomarker candidate has been detected.

A set of molecular assays for resistance mechanisms in *L. salmonis* has either just become available or are on the verge of being so. Figure 2 provides an overview of approaches to identify suitable molecular markers for resistance.

Dispersal and mixing of sea lice genes in the ocean

The geographic location of fish farms, even down to each net pen, has a major impact on the sea lice abundance in an area. As dispersal of infective larvae is largely dependent on water currents, mapping of the latter phenomena has been performed thoroughly in relation to sea lice dispersal and reproduction [21,45,46].

Salmonids are anadromous fish, and several of the species are potential hosts for sea lice. Farmed individuals are hatched in freshwater hatcheries, whereas wild individuals are hatched in fresh water, mainly in rivers, and live there for up to a few years before migrating to the oceans to graze. Those who avoid predators migrate back to the exact same river to spawn. Outgoing smolts may pass close to several fish farms on their way to pelagic or coastal waters, and are thereby susceptible to infestation by sea lice copepodids. These parasites are likely to be reproducing when the fish arrives in the open sea. Thus, the sea lice reservoir has historically been pelagic waters, at least for the Atlantic parasites. The migrating routes for Atlantic salmon are yet to be fully discovered. The less host specific *C. rogercresseyi* are thought to have their reservoir on other fish, mainly the Chilean rock cod [22]. In summary, the host conditions for sea lice differ significantly between locations, and are thus not really comparable.

Where do the sea lice come from?

As reviewed by Todd [47] and Costello [10] in 2006, no distinction of genetic material could be made between sea lice on wild, living salmonids and sea lice parasitizing farmed fish. In Western Canada, returning salmon are considered the major reservoir for new infestations [48]. On the other hand, two reports from 2008 and 2009 pointed at farmed fish being responsible for the majority of sea lice larvae in a Scottish sea loch [49,50]. Furthermore, as reviewed by Costello in 2009 [4], sea lice originating from fish farms may have a negative effect on wild salmon and sea trout populations. However, as reviewed by Torrisen *et al.* [6] and Jackson *et al.* [29], sea lice shed from farmed fish had little impact on the survival of wild salmonids, even though the infection pressure close to fish farms can be elevated several orders of magnitude [51]. All of this indicates, as claimed by Brooks in 2005 [21], that the infection pressure on both farmed and wild fish is site-specific, and that the larval dispersal varies considerably with local geographic and hydrographic factors. In general, local conditions determine the origin of the sea lice.

Current evidence supports the theory that several Atlantic sea lice populations are sharing genetic material coding for resistance, in this case resistance towards the compound EMB [38]. Following this argument, sea lice

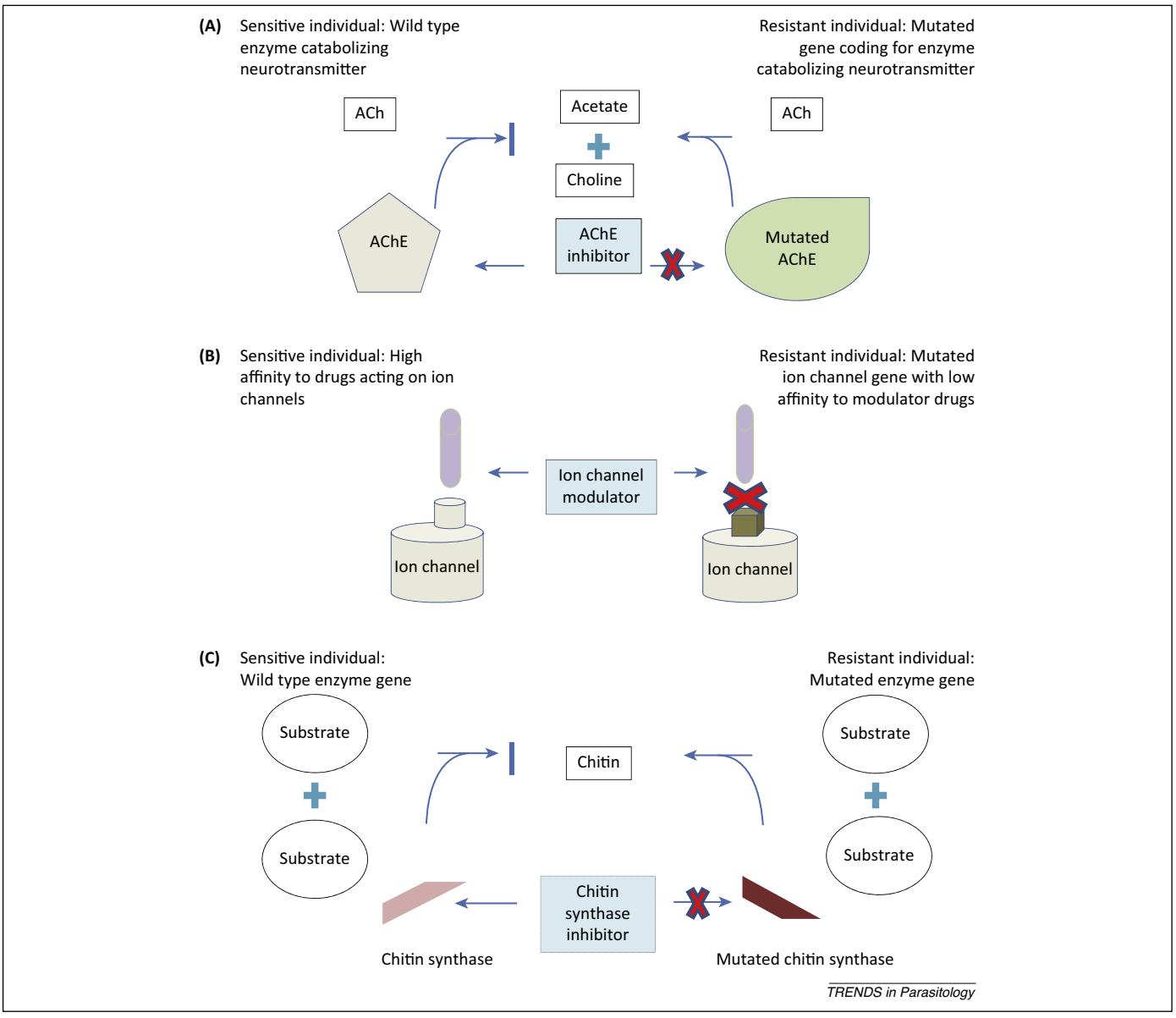

Figure 1. Frequent use of medicinal compounds will select individuals who possess features favoring medicine avoidance for the next generation. Such features, which are subject to molecular studies, are obtained by mutations from the wild type. In example (A) the mutated enzyme will not be targeted by the xenobiotic, and thus provide for effective neuronal transmission. (B) A mutated gene coding for ion channels responsible for neuronal signaling make the xenobiotic incapable of binding to its receptor, continuing the neuronal impulse in the organism. (C) Xenobiotics interfering with specific metabolic processes in target cells are ineffective in individuals possessing mutated enzymes, where physiological processes may continue.

insensitive to all the current remedies will most likely be prevailing in the Atlantic Ocean in the near future. This scenario would be regardless of the sea lice origin, either from farms self-infecting or from wild-salmonid colonization. In Pacific Canada and Chile, the situation is somewhat different, as the number of wild hosts in Pacific Canada is vast compared to Atlantic farming sites. In Chile, the host reservoir is thought to consist of several species as *C. rogercresseyi* is not very host specific. Thus, the better prognosis applies for the Western Canadian sea lice population, since resistance is less common there than in northern Europe and Chile.

Integrated pest management for sea lice

So far, every country has its own guidelines for sea lice regulation. As salmon migration routes are not clearly identified to date, a mutual, international set of sea lice legislation would be beneficial. The European countries could then be considered as one area. Dispersal of resistant parasites from Norway to adjacent countries would possibly negatively affect the industry. The Eastern and Western coast of North America would then be another two zones, with Chile, isolated geographically and carrying its own species of sea lice, should be considered the fourth zone.

Integrated pest management (IPM) principles have been important in salmon farming ever since Mordue and Pike introduced the concept in 2002 [52]. IPM encompasses several precaution measures to combat parasites. The Food and Agriculture Organization of the United Nations states: 'The careful consideration of all available pest control techniques and subsequent integration of

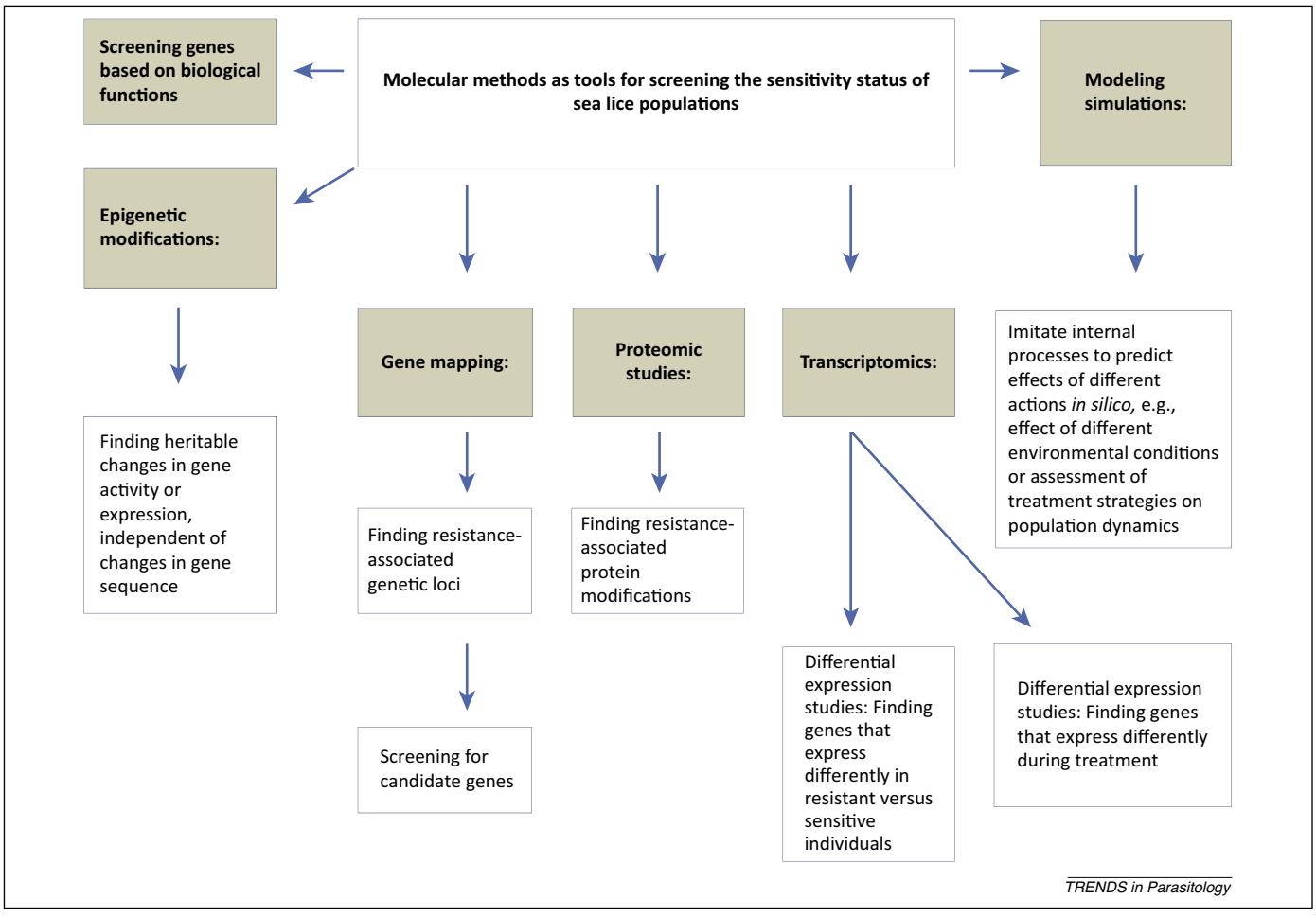

Figure 2. Flow chart visualizing areas of possible approaches in molecular biology-based research in resistance.

appropriate measures that discourage the development of pest populations and keep pesticides and other interventions to levels that are economically justified and reduce or minimize risks to human health and the environment. IPM emphasizes the growth of a healthy crop with the least possible disruption to agro-ecosystems and encourages natural pest control mechanisms' (http://www.fao.org/agriculture/crops/thematic-sitemap/theme/pests/ipm/en/). Brooks *et al*. [53] presented a similar concept in 2009, retrospectively reflecting considerable progress regarding measures taken by farmers and authorities 5 years later.

In a broader 10-year perspective, IPM should include more pathogens in the same program. For salmonids, this would include amoebic gill disease, virus-borne diseases such as pancreatic disease and infectious salmon anemia, which all have substantial impact on the fish and cause economic challenges for the industry [26] [Store Norske leksikon (https://snl.no/fiskeoppdrett)].

Future perspectives

The ongoing whole genome sequencing and annotation of *L. salmonis* and potentially also of *C. rogercresseyi* opens numerous avenues to develop new diagnostic methods, essential to combat resistance effectively. There are, however, challenges connected to such studies. Amidines, such as amitraz, have been used against ticks for more than 30 years. Still, the drug's mode of action is relatively unknown [54]. Similarly, the mechanisms connected to the pharmacology of EMB in sea lice are still subject to speculation [55]. This provides indications that investigating medicinal compounds, their effects on sea lice, and possible resistance mechanisms, are potentially substantial tasks to deal with. Mutations causing resistance may be appearing in nuclear DNA as well as in ribosomal or mitochondrial DNA, of which the latter in particular may represent hard fought discoveries.

Until novel medicinal compounds become available, salmonids infested with sea lice will to some degree have to be treated with compounds the parasites have developed some tolerance against. This will enhance the need for accurate and reliable diagnostic tests, such as qPCR. In this respect, molecular studies of all instars will be of crucial importance, in order to gain knowledge about remedies and their specific effect on defined life cycle stages. In particular, the expression of various proteins in the chalimus stage is less known, representing a potential for more effective extirpation of sea lice.

Sea lice management is subject to intense research and development in several countries. Currently, large resources are targeting a vaccine against the parasites in question, anti-attachment diets are in development, offshore farms are in the planning, whereas cleaner fish and laser treatments are already in use. Regarding medicinal compounds, additional substances with effects on one

or more parasitic stage could be introduced if discovered. Medicinal products ought to be the last preventive tool in line, instead of the first, which they traditionally have been. Altogether, these novel non-medicinal methods may have the potential to prolong the effect of the current medicinal compounds.

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