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Traceability of aquatic animals

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Summary

Effective methods of traceability are urgently required for use in research as well as in different types of aquaculture operations and to control trade in aquatic animals and products. In regard to the marking of fish, many different tagging methods have been described and the method to be used depends on the purpose and need for tagging. In contrast, for molluscs and crustaceans, only a few methods of marking such animals have been described, due to the practical difficulties.

The authors first describe the different methods for tracing fish and fishery products, by means of external tags, such as Floy tags, Carlin tags and passive integrated transponder tags; chemical marking using inorganic substances such as silver nitrate or potassium nitrate, pigments, oxytetracycline, etc.; and several different types of electronic devices in which basic information such as the strain of fish, farm of origin or weight can be stored. Genetic traceability using deoxyribonucleic acid profiling is developing quite rapidly for cultured brood stocks and wild populations. This technique may be used with very high degrees of confidence to assign to or exclude animals or products from their claimed origin, paternity or strain, and may be used as evidence in court proceedings.

The second section of this paper describes the traceability of live molluscs for restocking and for human consumption. In these applications, genetic markers have been demonstrated to be suitable. Mechanical tagging on a small scale for research purposes has also been used. Otherwise, the only means of tracing live molluscs are the movement documents and the labelling on boxes that certifies the origin of the commodity.

The third section describes the methods available for tracing live and dead crustaceans. A large variety of physical tagging methods for decapod crustaceans is described, such as the injection of biological stains (fast green, Niagara sky blue, trypan red and blue) and external tags such as coloured streamer tags, wire tags and a variety of anchor tags. Furthermore, a number of different internal coding methods, such as the coded micro-wire tags and injected elastomer tags are discussed in detail. As is the case for fish, genetic molecular techniques are also applied in population studies of crustaceans; some of the molecular genetic methods are described. Prawns for human consumption are most frequently packed whole or as tails after the necessary sorting, washing and freezing and the only way of performing a traceback is through documents relating to movement, invoices, health certificates and labelling of the boxes. The minimum requirements for labelling would be the content of the packages, i.e. species, quantity, identification of the manufacturer (name and address), packing place, importer/exporter or vendor of the product, in addition to the loading bill number.

Keywords

Aquatic animals – Crustacea – Fish – Genetic markers – Molluscs – Tags – Traceability.

– the above-mentioned authority must verify that the identification codes for equipment made available to veterinarians comply with code definitions, and that the transponder and the pre-printed identity card (containing the transponder number) are distributed together

– a link between the different registers must be created when an animal has two types of marking (tattoo and transponder)

– procedures must be established regarding the identification of domestic carnivores from other countries that remain on French territory for more than three months.

The owner of a domestic carnivore shall be able to choose, for identification purposes, between a tattoo and an electronic implant.

To ensure compliance with technical recommendations, specific regulations will specify procedures for manufacture, authorisation, marketing and use of electronic identification equipment, as well as sanctions that apply in the event that such regulations are violated.

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Introduction

Aquatic animals and aquatic animal products are among the principal sources of protein in the human diet. On a global scale, fish and fishery products are the most important source and constitute more than 80% of the total seafood produced. Thus, significant trade occurs in aquatic animals from wild sources as well as from aquaculture.

The tracing of aquatic animals or aquatic animal products to the initial source is much more difficult than for terrestrial animals and products. This arises from the methods of catching, handling and distributing fish from the oceans as well as aquaculture enterprises, the large number of different species and individuals, the physiological status of the animals and other related factors. In many cases, documents pertaining to movement and the name of the producer on the box(es) containing the aquatic animal/aquatic animal product may be the only means of identification. However, this will not be sufficient from a quality control point of view, as no guarantee can be given that the contents of the box really is derived from the source described.

Occasional 'accidents' in fish farms may result in escapees that may have an impact on wild populations. This problem has arisen in connection with Atlantic salmon farming in Norway; the proportion of escaped farmed salmon in some rivers has been reported to be as high as 80% (35). To monitor such escapees and identify the fisheries concerned, in addition to information on the size and cause of the escape, a tagging system that distinguishes between farmed and feral fish is also necessary. This is particularly important during the spawning season. The most commonly used type of identification has been fin clipping, but microchips have also been suggested as a possible identification method.

Tagging of farmed fish will continue to be a matter for discussion because of the associated costs and possible secondary effects, such as reduced growth, fish health aspects (secondary infections), etc. Questions regarding large-scale tagging of farmed fish have been considered in Norway by the Marine Laboratory/Norwegian Institute of Natural Resources, on the request of the Ministry of Fisheries and the Directorate of Natural Resources. The method most likely to be recommended is the snout tag. For the marking of farmed fish, approximately 3,000 different codes would be needed, and this would require research and development (35).

Several arguments for and against the use of this method have been addressed, and the Norwegian Fish Farmers Association has pointed out that both aquatic animal welfare considerations and the marketing aspects of the method must first be considered. Since the heads of fish also may be eaten, the potential risk to the consumer must be ascertained. However, tracing from the market back to the farm will become more and more important, and thus the method will fulfil the increased need for traceability in the future.

To ensure food safety and the interests of the consumer, a European Union (EU) Directive covering the trade of fish and fish products will be enforced in 2002, requiring that all fish to be sold by fishmongers or in supermarkets be tagged in a way that will allow traceback to the geographical location where each fish was caught or farmed.

Over the years, several different tagging devices have been developed for different purposes. The principles of the different identification systems and practical applications are described in more detail below.

Traceability of fish and fish products

Live fish (wild/farmed/escapees), aquarium fish and fish eggs

Tagging of live fish may be performed in many different ways, depending on the purpose of the tagging. For trade of fish derived from fish farming or otherwise, tagging is seldom performed on individual fish. The only methods of tracing such consignments are the movement documents (health certificates, transport documents, etc.) accompanying the commodity from the sender to the receiving farm (Fig. 1). However, deoxyribonucleic acid (DNA) fingerprinting, which has been developed for certain strains/breeds of given species, allows the tracing of the fish in question back to the source (see the subsection entitled 'Genetic markers').

Tagging on a large scale has been used for research purposes, such as population studies and feeding experiments, entailing the development of several different types of methods (35, 64).

A simple method of tagging is the use of different fin clipping patterns, but the development of various external mechanical tags, such as Carlin tags, Floy tags, anchor tags and visible implant tags (VIT) has meant that these have become the principal tagging methods. However, other means of tagging, such as coded wire-tags (CWTs), thermal branding (burning and freezing), chemical tagging, radioactive isotopes and genetic fingerprints have also been widely used.

Physical tagging

Fin clipping

The clipping of fins in different combinations and patterns for identifying or tracing the origin of individual fish has been used mostly under experimental conditions (e.g. vaccination experiments). Fin clipping may also be used in combination with other types of tagging such as CWTs and may thus be a valuable tool for mass tagging of fish for population studies, etc.

MOVEMENT DOCUMENT FOR LIVE FISH, EGGS OR GAMETES FROM AN APPROVED FARM

I. Country of origin _____

II. Farm of origin (name and address) _____

III. Animals or products _____

IV. Destination

Country of destination _____

Consignee (name and address) _____

V. Means of transport (nature and identification) _____

VI. Health attestation

I, the undersigned, hereby certify that the animals or products forming the present consignment originate from an approved farm and that they satisfy the requirements of Directive 91/67/EEC.

Issued at: _____ , date: _____

Name of official service _____

Stamp of official service _____

Name (in capitals) _____

Function of signing officer _____

Signature _____

Fig. 1
Information to be given in a European Union document for the movement of live fish, eggs or gametes from an approved farm

However, regeneration of fins may cause problems in regard to identification (39). The adipose fin does not regenerate and thus adipose fin clipping is a good choice. Adipose fin clipping may also be used in combination with CWTs.

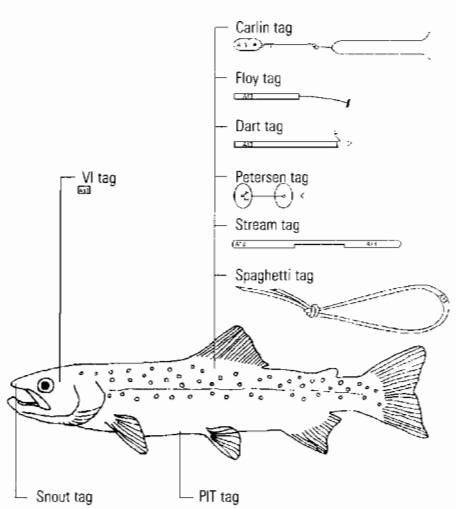
The advantages of fin clipping are that the mark is permanent if performed properly, and very visible, and the procedure is inexpensive. The disadvantages are the limited numbers of combinations, the regeneration of the fin that renders correct identification difficult, as well as increased mortality in the fish. Sockeye salmon (*Oncorhynchus nerka*) reportedly suffer higher mortality when fin clipped, compared to those marked with tetracycline (72).

External tags

External marks on animals have been used for several hundred years to enable the origin of a given animal to be traced. According to Heggberget *et al.* (35), the earliest description of tagging fish was in 1653, as reported by Walton and Cotton in 1898, referred to by McFarland *et al.* (47). Many of the different marks were used to study migration, growth and mortality, including catching rates, of important fish species such as salmonids.

The external marks may have different features and may be attached to the fish in different ways. One of the most common types is a small metal plate, or now more often a plastic plate, with an inscription giving instructions of where to send information about the catch.

All external tags require attachment with threads, wires or filaments, and since these tags perforate the skin and musculature, external tags have the disadvantage of giving rise to lesions in the skin and musculature, thus causing secondary fungal infections and algal attachments to the wounds. Reduced growth may also be observed (53). Thus, external tags are not suitable for fish farming purposes (Fig. 2).



VI : visible implant
PIT : passive integrated transponder

Fig. 2
External tag device used for fish
Source: Barlaup and Åtland (3) as shown in Heggberget *et al.* (35)

Carlin tag

The Carlin tag consists of a small tag with an alphanumeric code attached to the fish by means of two metal strings, or now more often a polypropylene thread, perforated through the dorsal musculature and joined together on the other side of the fish (Fig. 2).

Anchor tags

The Floy tag and similar tags from other producers, are inserted into the fish by means of an 'attachment gun' in the same area as described for the Carlin tag. As with the Carlin tag, the Floy tag also makes permanent wounds in the skin of the fish, resulting in secondary infections.

Thermal branding

Thermal branding of fish may involve either hot or cold branding tools which are effective and used widely. The retention of temperature-induced branding is due to disruption and uneven regeneration of scales (32), and the branding marks may be visible for as long as two years. Injuries due to hot branding are usually greater than those due to cold branding. However, hot branding marks develop more rapidly than those developed by freeze branding. Letters are usually not a good choice if a fish is likely to grow considerably (e.g. salmonids) as the symbols must be easy to distinguish after part of the symbol has grown out.

Hot branding

The method of hot branding involves heating metal that leaves a mark when placed on the skin. Several different means of heating the device have been described, such as boiling in water, electric wood-burning or soldering irons, lasers or electronic devices using NiChrome® wire as the heating element (17, 32). Branding of fish with laser beams has the disadvantages of high cost and the inability to produce a sufficiently accurate beam of light. Furthermore, laser equipment is reported to have mixed success under field conditions, because the power requirement is prohibitive for most field situations. In field trials in Columbia River, United States of America (USA), large holes developed in the bodies of laser-branded fish; this is not acceptable from an animal welfare point of view. Electronic hot branding equipment has a low power consumption, and is simple, portable, convenient and effective to use under field conditions. The thin filament of the nichrome can easily be shaped to create different code symbols and is suitable for pikeperch (*Stizostedion lucioperca*) and other species with small and firmly attached scales (67).

Freeze branding

Freeze branding in fish has been a tool used principally for research (feeding, genetic, behavioural studies, etc.) in experimental stations or in studies of wild stocks. Freeze branding has been used for several fish species including Atlantic salmon (*Salmo salar*) (43) and Atlantic halibut (*Hippoglossus hippoglossus*) (9).

Freeze branding may be conducted by using brands consisting of lead typewriter letters which are attached to wooden handles and cooled in a mixture of acetone and dry ice (23), or ethanol and dry ice (25), or by means of a permanent branding tool using liquid nitrogen (Fig. 3). Prior to branding, the fish need to be anaesthetised, after which the brands may be applied on different parts of the body for a few seconds (Fig. 4).

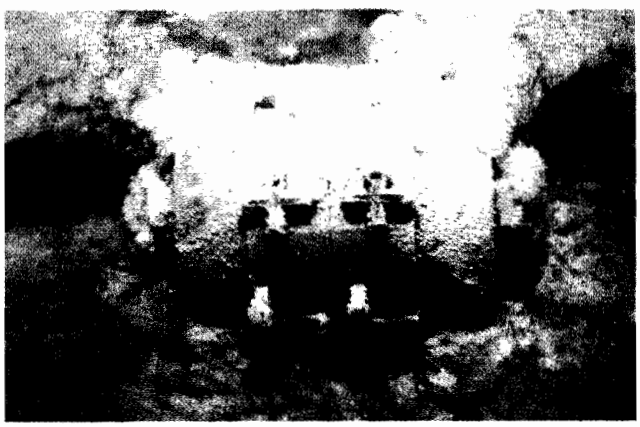


Fig. 3
Device for freeze branding of fish
Photo: courtesy of V. Vassvik

Fig. 4
Atlantic Salmon with freeze brands on the side
Photo: courtesy of V. Vassvik

Chemical tagging

Inorganic substances

Chemical branding of fish may be performed using silver nitrate or potassium nitrate. The brands are induced by chemical 'burning' due to application of a 2.0% solution of silver nitrate or a 2.5% solution of potassium permanganate. According to Myers and Iwamoto, the techniques used may vary and the authors report that chemical brands only produce temporary marks in juvenile tilapia (*Tilapia niloticus*) (54).

Pigment tagging (tattooing)

Jet injection of alcian blue in Atlantic salmon fins has proved to be a method which produces satisfactory marks (36). The readability of such marks after six months is still good, and if

remarking is employed, good marks can be maintained for an unlimited time. If remarking is not performed, fading of the colour will occur, depending on factors such as growth rate. However, under most growth conditions, satisfactory marks still exist after one and a half years. Other chemicals, such as hydrated chromium oxide, have also been used, but the visibility is lost after six months.

The two fluorescent chemicals alazarine complexone and alazarine red S have been applied for the marking of otholithes in cod (*Gadus morhua*) for population studies (13).

Panjet marks created by injecting dyes under pressure is another means of tagging fish (36), but the method is not suitable for fish for consumption.

None of the chemical marking techniques should be used for either restocking or for fish farming purposes.

Tetracyclines

According to Raymond (64) with reference to Weber and Wahle (72), tetracyclines are a promising fish marking method, not only as a permanent mark, but also as an excellent control for assessing fin-clip or other marking-induced mortality in hatcheries. Tetracyclines have also been used to mark fish eggs (35). Tetracyclines may create bands on the fish otholithes that can be later observed as a fluorescing layer or band with ultraviolet light.

In the opinion of the authors, the use of an antimicrobial drug for tagging purposes is unacceptable. Other disadvantages of the use of antimicrobials and tetracyclines are the lack of external signs of the tagging, the limited numbers of combinations and the fact that the fish has to be killed to identify the marking.

Internal tagging

Visible implant tags

The VIT is a small tag that is inserted into the non-pigmented area just behind the eye of the fish. In principle, the tag may be read without further intervention. The tag is inserted by means of an injector and, in addition to combinations of figures and letters, tags also have different colours. This increases the number of possible combinations. The disadvantages of using this tag are both that the tagging has to be performed manually and that the tag losses under experimental conditions have been reported to be high (>30%). One of the reasons for the loss of tags is that the tag works its way out of the skin or backwards into the fish, becoming invisible. However, the VIT has been reported to be used with good effect in several different species including rainbow trout (*O. mykiss*), Chinook salmon (*O. tshawytscha*), coho salmon (*O. kisutch*), pumpkinseed (*Lepomis gibbosus*) and orange trout darter (*Etheostoma spectabile*) (33).

Other tags using the same principles as the VIT are visible implant fluorescent filaments (VIF) and visible implant fluorescent elastomers (VIE), which are inserted into the same region as the VIT tag. Both tags are visible in fluorescent light, but while the VIF tag is 1 mm in length and 0.25 mm in diameter, the VIE tag is a polymer solution that stiffens shortly after injection. The loss of these tags is estimated at 20%, and due to the small number of different colours available, differentiation between different hatchery populations by colour is not possible.

Coded wire-tags

According to Heggberget *et al.*, coded wire-tags, usually known as CWTs or microtags, are the most suitable tag for both farmed fish and salmon for cultivation purposes (35). The CWT consists of a stainless steel wire that is introduced into the snout of the fish (thus the common name 'snout tag'), or in other parts of the body of the fish (Fig. 5). Such tags normally have a binary code, although the most recent tags have numeric codes etched on the wire. The tag has been used for many years in different parts of the world, especially for fish, but also for crustaceans (10).

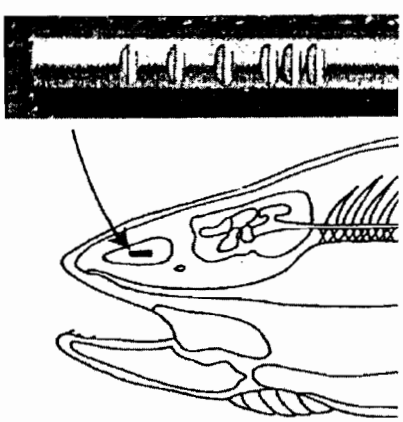


Fig. 5
Snout tag in fish
Source: Johnson (40) and Nielsen (56) as shown in Heggberget *et al.* (35)

In common with the VIT, a magnetic field induced on the tag can be used to detect the CWT. Automatic devices have been developed for detecting the presence of tagged fish, but the tags themselves have to be read manually to identify the code (51). The automated systems for tag recovery are generally used to sort the catch, especially when the catch is large. The fish, whether farmed or wild, may be identified to almost 100% accuracy, and thus separate stocks of fish can be differentiated. This may be important under several circumstances, such as identifying escapees from farms, fish used for stock enhancement and for fish farm husbandry purposes.

Solomon describes the feasibility and advantages of tagging salmon using CWTs (70, 71). Product traceability of salmon

would be a major advantage to the fish farming industry in several respects. Animal welfare issues may be another important issue but, to date, histological examinations have demonstrated no adverse tissue reaction to the presence of the tag and no effects upon survival, growth rate or behaviour.

Furthermore, the CWT could be used to indicate the vaccination status if vaccination and tagging were performed simultaneously.

The disadvantages of the CWT are mortality due to secondary infections, and reduced growth due to destruction of tissues in the snout area which reduces the ability for food intake (4). However, according the Bergman *et al.*, the use of CWTs does not create extensive damage in the snout area, either macroscopically or microscopically (histology) (10), and losses of this type of tag have been reported to be minor (12). However, Morrison and Zajac report that CWT has had negative effects in chum salmon (*O. keta*) (52).

Electronic tags

The passive integrated transponder (PIT) tag is an electronic tag which has been developed for fish (63) and which may be inserted into the abdominal cavity or various other parts of the body. A reader is used to activate the transponder and to identify the tag by means of the signal emitted.

If data relevant to an individual fish (strain, weight, length, farm, tank, etc.) is recorded on an electronic file when tagging is performed, and later combined with data collected from the reader and linked up to a computer program, all information available for the fish in question can be easily accessed on the server. This method has been used in various types of fish experiments such as behavioural studies, growth studies, migration studies and infection experiments, but may also be of use in the more advanced fish farm operations to follow the development of the fish population in the tanks, ponds or cages, and to trace escapees back to their origin.

Within breeding stations, electronic tagging has been introduced to establish a reliable identification of selected progeny from electronically tagged brood stock. By introducing this principle, which gives optimal identification, as many families as required can be kept in the breeding pyramid. This means that different types of fish can be developed more readily for different markets to satisfy the requirements of the customer.

Tagged fish are usually not affected to a great extent and the wounds connected with the introduction of the tag into the belly heal rapidly without secondary problems of any kind. Regular reported reading of the tags does not appear to adversely affect the fish to a great extent.

Tags containing a radio transmitter may be used for migration studies of fish returning to spawn.

Several different commercial tags exist such as 'Trovan' and 'Unique 2100'. These tags may in principle be used repeatedly if identified during the slaughter process, but costs will nevertheless be high (35).

Biological tagging

Natural parasites

The parasitic fauna of a fish may indicate the origin of a fish population; for example, if a certain parasite in the fish has a restricted geographical range, traceback to a certain region or country may be possible, based on parasitological findings.

Genetic markers

Genetic marking may be used if natural differences exist between groups of fish. Over the last few decades, biochemical and molecular markers have been used increasingly to study genetic diversity (21), and new genetic markers and statistical analyses have made multilocus genotyping a very valuable tool. In recent years, genomic programmes in a series of species, coupled with powerful post genomic tools (bioinformatics), DNA-chip based arrays and industrial outlets (e.g. superior brood stocks and novel drug design), have totally changed the scope and opportunities for molecular tracing of biological products, including food authenticity. As in the classical human forensics field, which has been strengthened by DNA profiling to provide powerful evidence in court proceedings, samples from food, cultured brood stocks or wild populations may be assigned to, or excluded from, a claimed origin, paternity or strain with a high degree of certainty.

Several approaches exist to employing molecular techniques and strategies for tracing biological resources and products, a prime example being DNA profiling, which is the modern version of blood typing. The classical blood group and biochemical polymorphism typing used in human forensics, paternity tests for the livestock industry and wild strain population genetics and management, is less conclusive and has a lower resolution power than the current DNA technologies. In addition, DNA profiling offers totally new opportunities in terms of applying automated electronic reading systems with high throughput cost-saving capacities. The DNA profiling approach is based on the simple principle of similarity and differences (i.e. genetic variation), comparing individuals, families, stocks/strains or species. Since such similarities and differences are reflected in the genes, they can be identified and displayed as unique DNA profiles. No individual is identical to another individual, except for identical twins. Population, stock or strain profiles can be achieved by two approaches, as follows:

a) the individuals are compared by genotype in a cluster analysis and those which group together are considered as homogenous and unique strains with a certain genetic distance from the other clusters or strains

b) populations are characterised by gene frequencies (allele frequencies at all involved loci), and grouping and distances are calculated on the basis of such frequency patterns.

In theory, and also for most practical purposes, such profiles can distinguish families and strains at a statistically significant level and can also assign or exclude individuals to a family or strain. To enable individuals or samples to be assigned to strains or stocks, which is highly relevant to the tracing of the origin of food, whether meat, fish or plant products, three prerequisites exist, as follows:

a) a significant genetic distance exists between the stocks or strains under examination (e.g. Norwegian Red and Hereford cattle, GenoMar super tilapia and other tilapia strains)

b) genetic profile databases have been developed and maintained for the strains in question

c) a sufficient number of genetic systems (e.g. chip-friendly markers such as single nucleotide polymorphisms [SNPs]) are available for testing to ensure sufficient statistical power.

As an example of the latter, with twenty SNP loci, the likelihood that two randomly sampled individuals from an out-bred or a wild population will have the same genotype is approximately 1:100 million (the figures will depend on gene frequencies) and the power of exclusion in a paternity test is 99.8% (on average 99.8 out of 100 wrong parents are disclosed by the test). Other genetic systems or markers such as microsatellites (length polymorphism) are also available and suitable for DNA profiling. The different systems have both advantages and disadvantages with respect to the quality of information (statistical power in the analysis of the data) as well as ease of typing. Microsatellites generally have a higher degree of informativeness (higher number of alleles per locus) compared to SNPs (which mostly display a wild type and a mutant). However, the latter genetic markers are more stable (fewer mutations) as well as being far more convenient for automated, high throughput typing. Hence, for typing of a large number of individuals or samples, it is imperative to choose genetic markers and technologies that are robust, capable of automation and cost-efficient; SNPs mounted on DNA-chips fulfil these criteria.

In conclusion, modern molecular techniques combined with sufficiently developed reference genetic databases and modern transport logistics now offer tools which enable samples of food or individuals to be assigned to or excluded from their genetic origin with high statistical power. In addition to food security, these tools are also highly relevant to the commercial protection of genetically advanced brood stocks and food trade marks or brands.

Fish for consumption

Whole fish (fresh or frozen)

Identification of fish and fish products for human consumption is usually based on movement documents, trade marks on boxes and other types of package labelling (e.g. vacuum packed products), gill cover tags, etc.

In addition to the need for traceability of live fish as described above, modern industrial aquaculture also requires a means to control production, planning of future production and optimisation of operations at the farm level. This includes all biological information from brood stock to slaughter. Several systems exist for the tracing and documentation of the production process from brood stock and egg production to the final commercial product. 'Superior tracing' is one of the tools available which facilitates the documentation required through the production cycle and which belongs to a collection of Windows™-based information technology solutions for management in aquaculture (O. Jamtøy, personal communication).

Traceability of exports of fish and fish products may also be improved by this type of system in that the fish and fish products may be traced back to a farm of origin if a complaint is received from a customer.

Many of the leading exporters of farmed salmonids in Norway have established a system of data recording for the product being sold. This system allows the traceback of fish to the hatchery that produced the eggs, and identifies hatching date, date of stocking in sea water, licence number of farm site, cage number and date of slaughter. In addition, the system includes the average weight of the fish when stocked into sea water, average weight at slaughter, harvest loss, feeding regimes (with specification of type of feed and pellet size at different periods during rearing), vaccination and medical treatment given. The input screen for this data recording is presented in Figure 6.

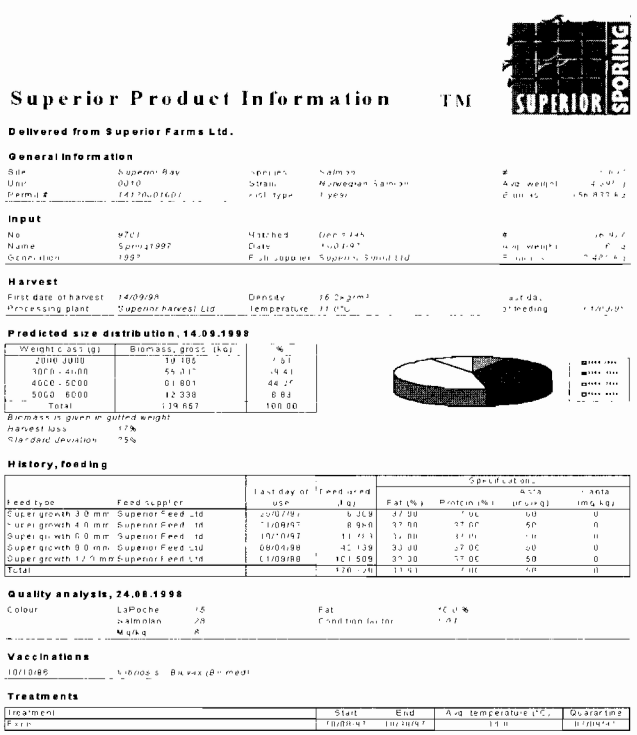


Fig. 6
Example of data recording using 'Superior Product Information'

After slaughter, fish are normally packed directly into polystyrene boxes and, in some cases, may have gill cover tags, which indicate the source of the product. The boxes are marked with data including the name of the slaughterhouse, destination and freight bill number, as well as the trademark of the exporting company. Figure 7 shows boxes ready for transportation.

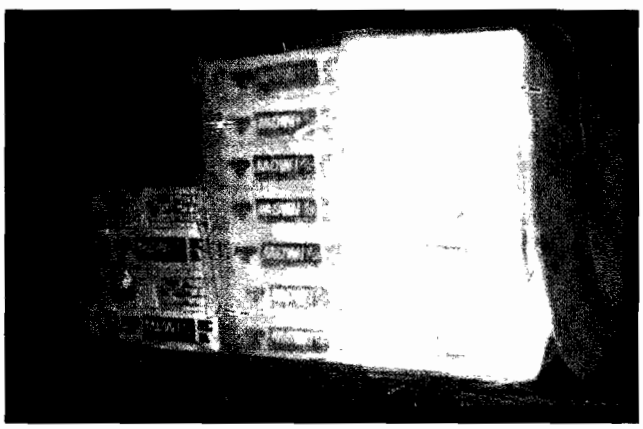


Fig. 7
Polystyrene boxes used in transport of fish for consumption
Photo: T. Håstein

Processed products

Salmonid fish for consumption is often processed into different products such as smoked salmon, marinated salmon or salmon steaks. The traceability of such products depends on the bookkeeping of the processing industry in regard to the origin of the fish processed, because the different types of products usually only show the trade mark of the producer when presented for human consumption, although, in some cases, the country of origin is also stated.

Traceability of molluscs

Mollusc farming is traditionally based on wild stocks and natural populations that frequently do not meet market demand, usually because of low quality products, over-fishing of the resources, or disease outbreaks. Until now, the answer to this has been the introduction of new stocks or new species (31). However, these introductions are limited by the availability of suitable species. Over the past decade, the aquaculture of molluscs has been growing at an average annual rate of approximately 11%, and in 1997, the total global mollusc aquaculture production was 8.5 million metric tons, which represents a 23.8% contribution to total global aquaculture production. The ecological impact of introduction of pathogens through movements of farmed molluscs appears to be an important underlying cause of losses currently recognised as a significant constraint to mollusc production and trade (11). Furthermore, in a large majority of cases, molluscs are marketed alive and are often consumed raw. Human health problems may occur after the placing on the market of live molluscs; traceback to the establishment of despatch and harvesting area of origin is thus important. Consequently, tracing of transfers and movements is of prime importance both from an aquatic animal and human health point of view.

Live molluscs for restocking

Genetic markers

Aquaculture of molluscs is based on a limited number of species belonging to four main groups (oysters, mussels, clams and scallops). The correct management of genetic resources is of great importance to ensure the sustainability of the mollusc industry. In the case of edible oysters, more than 95% of the world production is supplied by a single species, the Pacific oyster (*Crassostrea gigas*), and in numerous cases this oyster has been introduced to areas where it is a non-native species. Shell morphology is highly variable depending on growing conditions, and species determination is often unclear. Furthermore, inter-specific hybridisation is possible (26). Therefore, the development of species-specific genetic markers is useful for identification. These markers may be used to discriminate morphologically plastic species in the field (34), to trace the origin of non-native stocks (14), or to study the host-parasite interactions among broadly distributed populations of molluscs (16).

An accidental introduction of cupped oysters (*C. angulata*) from Portugal occurred in France after a vessel sunk in the Gironde estuary in 1868. As a result, aquaculture of cupped oysters in the region was extremely prosperous until the early 1970s when mass mortality occurred in *C. angulata* due to viral infection, leading to the disappearance of this species from the area by 1973. The Pacific oyster, which was introduced from Canada and Japan for restocking in 1971, is thought to have been illegally introduced by farmers previously, resulting in the decline in production of *C. angulata* in France. The two species are fully inter-fertile and morphologically indistinguishable. Genetic markers have been used to trace the origin of these non-native stocks of *C. angulata* and *C. gigas*. Recent results have revealed that the conspecific species, *C. angulata*, originated from Taipei China, and is distinguishable from *C. gigas* based on mitochondrial and genomic markers. Similarly, allozymes may appear as neutral and effective markers for tracking population bottlenecks in hatchery breeding production and reseeded populations. This has been successfully used during large-scale out-planting of red abalone (*Haliotis rufescens*) in southern California (27).

Physical tagging

Physical tags have sometimes been used for experimental studies. The movements of cuttlefish (*Sepia officinalis*) in the Gulf of Tunis were monitored by employing plastic fanion tags inserted in the anterior portion of the mantle (24). The tracking experiment lasted approximately five months. Similarly, plastic

stickers may be fixed on the surface of the shells of molluscs, providing an external and individual mark to trace animals and their progeny (useful for breeding programmes).

Live molluscs for consumption

Transport documentation

As stated earlier, traceback to the despatch and harvesting area of origin is important in the event of a human health problem after the marketing of live molluscs. Possible hazards may be pathogens from the harvest area, but natural bio-toxins, and chemical contaminants (28) can also present a risk to the consumer. For this purpose, regulations for registration and labelling systems must be introduced to ensure that the route of a batch can be followed after harvesting. Labelling of individual molluscs is almost impossible to achieve except for experimental purposes. A registration document may be issued by the competent authority for the identification of batches of live bivalve molluscs during transport from the production area to a despatch centre, purification centre, relaying area or processing plant. Such a document should state the identity and address of the gatherer, species and quantity of shellfish, date of harvesting, location of the production area, health status of the production area, approval number and place of destination for wrapping, relaying, purification or processing. The competent authority is then required to keep a register indicating numbers of registration documents, together with the names of the people collecting live molluscs to whom the documents were issued. If a production or relaying area is closed temporarily, the competent authority may then cease issuing registration documents for that area and suspend validity of registration documents already issued. Such considerations were taken into account in the EU regulation stipulating the health conditions for the production and placing on the market of live bivalve molluscs. Furthermore, at the farm level, registration by an official service of all farms rearing molluscs has been proposed. This registration should be kept updated regularly by recording live molluscs entering the farm, including information relating to the delivery, number or weight, size and origin. Similarly, any molluscs leaving the farm for re-immersion should be documented. This record should be kept for several years and open to scrutiny by the official authorities.

Processed molluscs

Apart from the labelling of the cans, boxes, etc. of processed molluscs, the product cannot be traced back to the original source of production, as the content of a given container may be derived from several different sources.

Traceability of crustaceans and crustacean products

Shrimp, lobster, crab and crayfish harvested from wild fisheries or derived from aquaculture constitute an important group of aquatic animals. Virtually all commercially valuable crustaceans are members of the order Decapoda. Whether consumed locally or traded internationally, this group of decapod crustaceans is among the most highly valued aquatic animal products. Consumer demand has historically outpaced the available supplies of these crustaceans from natural habitats, resulting in high prices for many of the favoured decapod species. This market demand has led to attempts to culture those species with the highest market value and those species which were amenable to captivity and culture from a biological and behavioural point of view.

The culture of crustaceans has expanded from isolated experimental projects to investigate the culture of a variety of species, into a major aquacultural industry that farms a number of decapod crustacean species. While technologies have been developed for the culture of the clawed lobsters of the genus *Homarus*, the culture of these species has not proven to be economically feasible. However, profitable methods for the culture of other decapod species have been developed, and industries that culture marine shrimp (family Penaeidiae), certain freshwater crayfish (families Astacidae, Cambaridae and Parastacidae), and freshwater prawns (family Palaemonidae) may be found in many countries. The most significant developments in crustacean aquaculture have been those involving marine penaeid shrimp and prawns. Farming of the marine penaeid shrimp has grown into an industry with an annual production of approximately 800,000 metric tons. The high value of penaeids places this product among the most economically important of current aquaculture industries. Given the value and the size of the capture and culture industries for shrimp, the following review of the methods used for tagging and traceability will concentrate on the penaeid shrimp.

Tagging of live crustaceans

Mutilation and staining methods

Many of the methods used to mark shrimp have been adapted from fish and some had also been previously used for lobsters. The problems encountered in marking crustacea, especially shrimp, are numerous, and in the earliest studies, methods developed for fish were found to be unsatisfactory because of the small size of shrimp, the periodic moulting of the exoskeleton, and because the attachment of tags or injection of foreign materials usually has some undesirable effects. Tagging by mutilation consisting of removal of appendages (i.e. uropods, pereiopods, chelipeds or the rostrum) provides a method for short-term marking of decapods. The method has been investigated in a variety of decapods such as shrimp (55), crayfish (73), clawed lobsters (46), and rock lobsters (75). However, because infections often result and because excised appendages are regenerated within a few moult cycles, this method of tagging is seldom used. Similarly, numbers or other marks may be applied using hot branding (46), or the application of water insoluble inks, paints and glues to the

exoskeleton of decapods, but these marks are lost with the cast exoskeleton during ecdysis (55, 73).

A large variety of mechanical tagging methods has been used by fishery biologists and managers in studies of wild decapod crustaceans (55). One of the first marking methods used in large-scale population studies of shrimp was the injection of biological stains (49, 65). Dyes such as fast green, Niagara sky blue 6B, trypan red, and trypan blue were used in large-scale field studies to mark juvenile shrimp (55). In population studies of wild penaeids in the Gulf of Mexico, recoveries as high as 62% were obtained for shrimp released after being tagged by injection with 0.03 ml to 0.3 ml (depending on the size of the shrimp) of 0.5% aqueous fast green. Although shrimp were injected in the tail muscle, the dye was rapidly absorbed and accumulated by tissues in the gills, giving the gills a distinct green colour that remained visible for weeks to months. No adverse effects of the dye on injected shrimp were noted. The colour faded gradually over two to three months, limiting the useful duration of the mark (55). Niagara sky blue dissolved 0.25% in water and injected (0.03 ml to 0.30 ml per shrimp, according to size) imparts a vivid blue colour to the gills of marked shrimp, and in field trials, up to 30% of those released were recovered at a later date. Trypan blue and red were investigated as marking materials for shrimp. Trypan red was found to have little value as a marking tool, as the reddish colour imparted to shrimp gills was difficult to distinguish from natural red and pink colours present in the gills and carapace (gill cover) of wild shrimp. Trypan blue when used as a 0.25% aqueous solution and injected into the tail (0.03 ml to 0.30 ml per shrimp, according to size) produced a lasting blue mark in the gills. However, trypan blue and red were found to be more toxic to shrimp than dyes such as fast green. In field trials, the highest returns of shrimp marked with trypan blue were 35% (55).

A variation in the use of internal dye markers in shrimp is the combination of petroleum jelly and biological dyes. When such mixtures are injected into the tail muscle of the fifth or sixth abdominal segment, the dye gradually moves from the site of injection to the gills over a period of several weeks. When mixed with petroleum jelly, fluorescent pigments such as saturn yellow, blaze orange, arc yellow and neon red were tested and found to work well as internal tags. Shrimp tagged with any of the dyes were easily detected under ultraviolet light (42, 55).

Spraying with fluorescent pigments has been used to mark shrimp and crayfish (7, 15). The animals are sprayed with pigment using a compressed-air paint-sprayer. Pigment particles are embedded in or penetrate the exoskeleton and are visible under ultraviolet light. Although large numbers of animals of various sizes can be tagged easily using the spray method, most of the pigment is lost during ecdysis, thus seriously limiting the use of this tagging method (7).

Staining of crustaceans by immersion has been used to mark large numbers of small shrimp for population studies. Northern brown shrimp (*Crangon crangon*) were successfully marked for short-term population studies by immersion for 3 min to 5 min in a 0.10% solution of methyl violet. In shrimp, the stain faded after several weeks, and all traces of the stain disappeared when the animals moulted (50). Immersion staining methods using biological dyes and food colourings were also examined by Wheeler for use in marking large numbers of post larval (PL) penaeid shrimp (74). Wheeler found that immersion of PL shrimp for 10 min in red and blue food colourings stained the digestive tract heavily enough to allow recognition two to three days later. Immersion of PL shrimp in solutions of Nile blue A and neutral red produced staining of the abdominal tissues which was visible for seven to fourteen days (74).

External tagging methods

Several types of external tags have been used successfully for shrimp and lobsters. Juvenile lobsters (*Homarus gammarus*) tagged through the abdomen with coloured streamer tags showed 97% survival and 100% tag retention nine months after tagging. A disadvantage of streamer tags noted in the lobster study was that sometimes the tags interfered with moulting (46). Streamer tags have been tested in penaeid shrimp using the polyethylene mini-ribbon tags (Floy Tag Company), which were inserted with a needle through the abdomen between the first and second abdominal segments (37, 48). To reduce infections caused by tagging, Marullo *et al.* investigated the practice of administering an antibiotic during the tagging operation (48). Howe and Hoyt found that although tag retention was high, mortality rates of shrimp tagged with these streamer tags was approximately 50% higher than the untagged controls (37). In these studies, mortality was higher among tagged animals because tags evoked attacks from untagged conspecifics and (in one trial) from fish.

The development of easily visible tags has provided information on growth rate, moult frequencies and mortality rates as well as lobster movement distances (68). The Burnham lobster tag appears to be the most suitable tag available for long-term studies on lobster migration (6).

Wire tags, Petersen disk tags, and a variety of anchor tags have been used with mixed success in studies of juveniles and adults in several species of penaeid shrimp. The Petersen tag, consisting of individually numbered, coloured (red, white or green) celluloid disks of 0.25 mm thickness and approximately 8 mm in diameter, with a central hole, were attached to shrimp with a nickel wire pin inserted through the musculature of the first abdominal segment (Fig. 8). One disk was placed on each side of the shrimp, and the pointed end of the pin was bent into a loop so that the disks could not be lost (55). In one large field study, the Peterson tag was used to determine the migrations and growth rates of white shrimp (*Penaeus setiferus*) along the southern Atlantic coast and the Gulf of Mexico coast of the USA. Of a total of 45,022 shrimp tagged and released, 7,167 or 16% were recovered (45).

Fig. 8
The Petersen tag shown as components and inserted through the abdomen of a juvenile Gulf white shrimp (*Penaeus setiferus*)
Photo: courtesy of the National Marine Fisheries Service, Galveston, Texas

Silver wire tags with small plastic disks have been used for mark/recapture studies of northern brown shrimp. A silver wire, 0.18 mm in diameter, carrying a numbered plastic disk of 6 mm in diameter, was wrapped around the junction of the cepahalothorax and abdomen. As the exoskeleton splits at this location during ecdysis, the tag was not lost or interfered with at moulting, and moulting was not affected (50).

Dart tags were shown to have limited value when used in studies with southern rock lobsters (*Jasus novaehollandiae*). Dart tags used in field studies were unsuccessful because of poor (19%) tag retention. Tags were dislodged at moulting, the nylon shafts broke, and lasting deformities and necrosis resulted at the insertion site on the abdomen (75). The use of anchor-type tags has been considered successful in the penaeid shrimp and in prawns. Bearden and McKenzie reported the use of Floy internal anchor tags in white shrimp (*P. setiferus*) (5), while Penn *et al.* reported on the successful use of toggle and Atkin tags in studies on the survival, growth and reproduction of western king prawns (*P. latisulculatus*) (61) and (*Metapenaeus macleayi*) (66) in waters of Australia.

Eye tags are commonly used to mark adult penaeid shrimp in captive brood stock populations to trace individual shrimp (Fig. 9). Numbered small plastic or metal bird leg bands are used for this purpose. The band is placed on the eye stock behind the bulbous portion of the compound eye. Such bands are tolerated well by shrimp and are not lost during ecdysis. However, this tagging method is applicable only to very large adult shrimp (30).

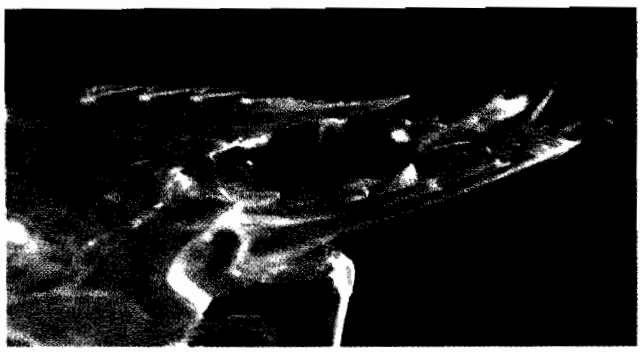


Fig. 9
'Eye tag' in a subadult *Penaeus vannamei* consisting of a numbered plastic leg band manufactured for use as a bird leg band
Photo: courtesy of S. Moss, Oceanic Institute of Honolulu, Hawaii

Internal tagging methods

Coded wire-tags

A number of internal coded tagging methods have been developed for use with decapods. One of the earliest methods used was the surgical insertion of numbered, coloured, polyvinyl chloride tags into juvenile penaeid shrimp (Fig. 10). These tags were 2 mm x 5 mm x 0.25 mm in size and were inserted using fine forceps through the thin cuticular articulation membrane between the first and second abdominal segment into the abdominal musculature just under the exoskeleton. Approximately 20,000 shrimp were marked using this tag in combination with a biological dye and released in three separate field studies; returns of marked animals from the fishery were approximately 4%. Mortality of laboratory-held shrimp with this tagging method was no more severe than that associated with the petroleum jelly/pigment mark alone (55).

Fig. 10
Insertion of a numbered internal plastic tag into the subcutis and muscle of a juvenile Gulf white shrimp (*Penaeus setiferus*)
Photo: courtesy of the National Marine Fisheries Service, Galveston, Texas

Yano *et al.* implanted short pieces of coloured fishing line under the ventral cuticle of the abdomen using a tuberculin syringe, the plunger of which was fitted with a 0.3 mm stainless steel wire to expel the fishing line fragment into the tissue (76). Tag retention and visibility were reported to be good and the tags induced no apparent detrimental side-effects. Similar coloured (brown, dark blue and green) pieces of fishing line injected using the method described above were used to mark two species of crayfish (*Cambarus laevis* and *Orconectes inermis*) found in wet caves in the USA. Crayfish marked with the coloured tags were also marked by a mutilation tag consisting of the removal of a uropod, and were numbered on the carapace with waterproof ink. Of 300 crayfish tagged, released and subsequently recovered after two to three and a half years, tag retention was 99% (73).

According to the literature provided by a supplier of CWTs, these tags have been used successfully to mark juvenile and adult stages of a wide variety of decapod crustaceans (Table I) (57).

Table I
Examples of successful applications of coded wire tags in small juvenile to adult size decapod crustaceans

Common name	Scientific name
European lobster	*Homarus gammarus*
American lobster	*Homarus americanus*
Snow crab	*Chionoecetes opilio*
Blue crab	*Callinectes sapidus*
Mud crab	*Scylla paramamosain*
Dublin Bay prawn	*Nephrops norvegicus*
Red swamp crayfish	*Procambarus clarckii*
Spot prawn	*Pandalus platyceros*
Northern brown shrimp	*Crangon crangon*
Kuruma prawn	*Penaeus japonicus*

The CWT is a small length of stainless steel wire (1.0 mm x 0.25 mm) which is magnetised and injected into the animal. Batches or individual tags are coded with a series of binary codes etched along the length of the tag. When used, tags are cut from a roll, magnetised, and using a patented system, injected into a haemocoel sinus, usually in the head of decapods such as shrimp and lobsters. Depending upon the decapod species, approximately 500 individuals may be tagged per hour. Studies of several species of decapod crustaceans have indicated that the tag has virtually no effect on growth and survival, and is reliably retained through moulting (Table I). Very small juveniles may be tagged and tag retention rates are very high; 100% is not unusual. Tagged animals are identified using magnetic detectors, either manually with a hand-held detector or in a tunnel detector in applications such as in processing lines at packing plants for harvested animals. A tag passing through the detector generates a signal that can allow recovery by hand or the incorporation of an automatic diverter gate. The binary code on each tag recovered may be read under a low power microscope (57).

Coded micro-wire tags were compared to other tagging methods (streamer tags, injected coloured elastomer tags, hot branding and rostrum removal) in clawed lobsters (*H. gammarus*). The authors concluded that internal CWTs and implanted coloured elastomer tags provided the most suitable tags (46). In another study, 15 mm to 22.5 mm total length spot prawns (*Pandalus platyceros*) were tagged with CWTs in the thoracic sinus. No differences in growth and survival among tagged and untagged animals were noted during the 174-day test period, and tag retention was 95% (62). Small juvenile (20 mm-41 mm total length) crayfish (*Procambarus clarckii*), marked with CWTs and maintained for observation in laboratory aquaria, retained the tag for life (38). One significant concern over the use of CWTs is the potential of the tags to injure unsuspecting consumers who might ingest implanted tags.

Elastomer tags have been developed recently and offer a relatively simple and inexpensive option for tagging all sizes of crustacean from small juveniles to adults, without posing any hazard to consumers through accidental ingestion (Fig. 11). The elastomer tag consists of a two-part medical grade liquid mixture which, when mixed, hardens into a firm rubbery mass. The elastomer tagging mix remains liquid for several hours before fully curing after approximately 24 h. Four fluorescent and three non-fluorescent colours are available from a commercial supplier along with the apparatus used to inject the

Fig. 11
Internal coloured (green, red, yellow and blue) elastomer tags inserted into the subcutis and muscles of the ventral aspect of the sixth abdominal segments of juvenile *Penaeus vannamei*
Photo: courtesy of S. Moss, Oceanic Institute of Honolulu, Hawaii

tag mixture (58). The main feature of the injector is a syringe fitted with a 27-gauge needle. The needle is inserted through the thin cuticle of the fifth or sixth abdominal segment in an anterior direction into the subcuticular above the underlying musculature, and the elastomer is gradually injected as the needle is withdrawn (30). Implanted coloured elastomer tags have been used in field trials to mark lobsters (*H. gammarus*) released for stock enhancement (46).

Elastomer tags have become a commonly-used tag for identifying individuals or members of families in penaeid shrimp breeding programmes (30, 58). In shrimp, the tag is most typically implanted lateral to the ventral nerve cord under the thin cuticle of the ventral side of the sixth abdominal segment, where it may be easily visualised with normal light or detected with the aid of ultraviolet light. Combinations of coloured tags implanted at different sites in the same shrimp provide a large number of possible coding combinations. For example, when using only one tag per shrimp, twelve distinguishable codes are possible by using the four available colours implanted into three locations; when three tags are used per shrimp, sixty-four distinguishable codes are obtainable by implanting four colours in three locations (30, 58).

In studies in which juvenile and adult white shrimp (*P. vannamei*) were tagged using the elastomer method, tag retention was 99.9% in the juveniles and 100% in the adult shrimp after a ten- to fourteen-week period. During that time, the juveniles moulted seventeen to twenty-three times and the adult shrimp moulted an estimated five to seven times. Among the five colours used, Godin *et al.* found the red-coloured tag to be the easiest to identify, while the lime-coloured tag was the most difficult (30). Histological evaluation of the tag *in situ* showed only mild inflammation and scarring at the tag site.

A recent innovative tagging method for crustaceans has been the application of PIT (passive integrated microchip transponder) tags to individual animals. These tags have been used successfully in relatively large freshwater prawns (*Macrobrachium rosenbergii*) (18). In addition, PIT tags are being used to mark adult penaeid shrimp (*P. monodon*) in a selective breeding programme in Madagascar (M. Le Groumellec, unpublished findings). These tags are implanted into soft tissues under the exoskeleton. The tags are of the same type as those used in dogs and cats, and are implanted into the body of a prawn or shrimp using a large-gauge needle fitted to a syringe. The PIT tag is read *in situ* from an animal in hand or from distances of approximately 10 cm. The transponders are 14 mm x 2 mm in size, are not adversely affected by shrimp tissue fluids, do not illicit a significant inflammatory response, and are not lost during moulting. A portable tag reader generates an induction field that activates and powers the tag to respond by emitting a unique signal that is displayed on the screen of the reader. Billions of possible codes can be generated, and the reader converts each into a unique alphanumeric identifier (18).

Genetic markers

Molecular techniques are increasingly being applied to studies of the population structure of wild crustaceans and as markers for specific traits in penaeid shrimp breeding programmes. For example, the use of allozyme electrophoresis has been applied to population studies and to species differentiation. Glucose-6-phosphate isomerase allozymes were found to be more suitable for differentiation of the PL stages of the closely-related penaeid prawns of Australia, *P. esculentus* and *P. semisulcatus*, than were traditional morphological methods. More recent technological developments have shown molecular methods to be more powerful tools than allozymes in the study of crustacean population structure. Benzie *et al.* used mitochondrial DNA (mtDNA) restriction fragment length polymorphism (RFLP) to confirm the findings of earlier studies based on allozymes that showed the *P. monodon* stocks of west and east Australia to be distinct, and the mtDNA provided RFLP differences on an even smaller geographical scale (8). Similarly, differences in mtDNA RFLP amplified by PCR and then sequenced, revealed large genetic differences between the closely related penaeids *P. vannamei* and *P. stylirostris* (60). Differences in RFLP of PCR amplified 28S ribosomal DNA were used to distinguish between three species of spiny lobsters (*Panulirus argus*, *P. guttatus* and *P. laevicauda*) which have morphologically indistinguishable phyllosome larvae (69).

Garcia *et al.* investigated the use of three molecular genetic techniques, RFLP, random amplification of polymorphic DNA (RAPD) and allozyme variability, to search for markers that could be used to distinguish between domesticated breeding lines of *P. vannamei* (29). The mtDNA and nuclear DNA RFLP and RAPD provided more useful genetic markers than did traditional allozyme methods. Similarily, RAPD methods were applied to population studies on the blue shrimp (*P. stylirostris*) in the Gulf of California fishery in Mexico (2). Unique markers were identified that distinguished six different stocks of the species in the Gulf of California. The use of RAPD methods has also identified markers in different domesticated breeding lines of *P. vannamei* for susceptibility to disease caused by infection with infectious hypodermal and haematopoietic necrosis virus (IHHNV; a parvovirus) and *Baculovirus penaei* (BP; a baculovirus) (1).

Jones *et al.* recently reviewed the various methods used in selective breeding programmes of the penaeid shrimp farming industry (Figs 12 and 13) (41). Of approximately ten genetic methods used to develop molecular markers for shrimp populations, Jones *et al.* concluded that the use of microsatellite markers (tandem repeats of two to seven base pairs) is emerging as the method of choice for shrimp (41). This is not unexpected since the method is being used throughout most mature agriculture industry sectors in marker-assisted genetic selection. Microsatellite markers may be employed, as follows:

a) to track diversity or establish relatedness among individuals, families, or populations

b) as genetic tags for species or strain identification

c) for marker-assisted selection to identify markers for economically important traits (e.g. rapid growth or disease resistance).

Fig. 12
A bi-coloured American lobster (*Homarus americanus*) produced through a selective breeding programme at the Massachusetts State Lobster Hatchery for possible use as a natural tag in stock enhancement studies
Photo: D.V. Lightner

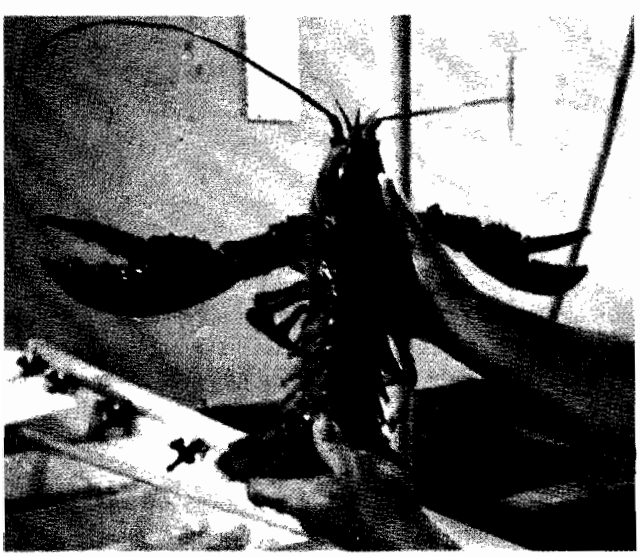


Fig. 13
A cobalt blue American lobster (*Homarus americanus*) produced through a selective breeding programme at the Massachusetts State Lobster Hatchery between 1960 and 1980, for possible use as a naturally tagged lobster in stock enhancement studies in New England
Photo: D.V. Lightner

Traceability of crustacean products in trade

Given the high value of crustacean products and because the principal markets for lobsters, crabs, shrimp and crayfish are dominated by relatively few affluent countries in east Asia, North America and western Europe, massive quantities of decapod crustaceans, particularly penaeid shrimp, are moved in international trade. Molecular methods hold great promise as tools that can be used to confirm the species and geographical source of live, fresh, or frozen crustacean products in trade. However, the exporters and importers of crustacean products still rely on the labelling standards of the Codex (20).

The general standards of the Codex for the labelling of foods are derived from the Codex Alimentarius Commission and are approved by all member nations of the Food and Agriculture Organization (FAO) and the World Health Organization (WHO) in accordance with the General Principles of the Codex Alimentarius (20). The requirements for labelling of food packages include the following:

a) name of the food (e.g. 'shrimp' or 'prawns', species, brand name, etc.)

b) quantitative list of ingredients

c) net contents and drained weight in metric units (for crustaceans, especially shrimp and prawns, this includes the 'count size', i.e. the number of whole shrimp or shrimp tails per pound, 100 g, or kilogram)

d) name and address of the manufacturer, packer, distributor, importer, exporter and/or vendor of the product

e) country of origin

f) lot identification (a code number that identifies the lot and the producing factory, packer, etc.), the date of processing or packing and storage instructions (19, 20).

Item *e*) is interesting in that this labelling requirement is often misused in the international trade of penaeid shrimp and prawns. Section 4.5.1 of the Codex general standard for the labelling of prepackaged foods reads 'The country of origin of the food shall be declared if its omission would mislead or deceive the customer'. However, section 4.5.2 of the same Codex standard states 'When a food undergoes processing in a second country which changes its nature, the country in which the processing is performed shall be considered to be the country of origin' (19, 20). For shrimp, the Codex authorisation to change the 'country of origin' label has been interpreted by the industry as requiring as little 'processing' as changing the shipping boxes and/or documentation that accompanies a shipment. This discrepancy has contributed to the practice of importation and re-processing of shrimp products from countries or regions where Office International

des Epizooties (OIE) notifiable pathogens, such as white spot syndrome virus (WSSV), are major problems in shrimp farms. Shrimp products with severe WSSV infections and which were subjected to 'emergency harvest' to reduce economic losses to farmers in the exporting country, have been imported and subjected to value-added re-processing in countries with no prior history of WSSV. The introduction and establishment of WSSV in the Americas in farmed or wild crab and shrimp stocks and in farmed shrimp may have occurred as a consequence of improper disposal of processing wastes resulting from this practice (22, 44, 59). Introductions of pathogens by this route would be less likely to occur if the Codex required the actual geographic or biological site of origin to be listed on all consignments of shrimp products moved in international trade, so that the authorities of importing countries might be better informed of the source and disease risks associated with particular types of imported shrimp products.

Conclusions

Tracing and identification of aquatic animals and animal products has been performed for many years and the methods used for different purposes have developed from primitive means such as fin clipping to more sophisticated methods such as CWTs and genetic markers, in parallel with developments in science.

Tagging has proven to be a useful tool in tracing a given commodity to the source, provided that the different methods used are applied in a transparent way.

Labelling of containers and accompanying documentation presenting details of the source, is still by far the most common method in use for international trade and is likely to remain so for the foreseeable future. However, such labelling is more open to abuse in terms of misrepresenting the true origin of the product, and as a result, more secure traceability methods are required for such trade.

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■

Traçabilité des animaux aquatiques

T. Håstein, B.J. Hill, F. Berthe & D.V. Lightner

Résumé

La traçabilité est devenue une priorité pour la recherche et les diverses activités aquacoles mais aussi pour le contrôle des échanges d'animaux aquatiques et de leurs produits. S'agissant du marquage des poissons, de nombreuses méthodes ont été décrites, suivant la finalité et les besoins auxquels elles répondent. En revanche, pour les mollusques et les crustacés, les procédés sont moins nombreux, en raison notamment de difficultés pratiques.

Les auteurs décrivent tout d'abord les différentes techniques de traçage des poissons et des produits de pisciculture : les marques externes telles que les marques Floy, les marques Carlin et les transmetteurs passifs intégrés ; les marques chimiques à l'aide de substances inorganiques comme le nitrate d'argent ou le nitrate de potassium, les pigments, l'oxytétracycline, etc.; et différents dispositifs électroniques permettant de stocker des informations de base comme la souche du poisson, l'élevage d'origine ou le poids. La traçabilité génétique grâce à la caractérisation de l'acide désoxyribonucléique se développe assez rapidement pour les élevages de reproducteurs ainsi que pour les populations

sauvages. Cette technique présente un degré très élevé de fiabilité pour confirmer ou infirmer l'origine, l'ascendance ou la souche des animaux ou des produits ; elle peut également servir de preuve devant les tribunaux.
La deuxième partie est consacrée à la traçabilité des mollusques destinés au repeuplement ou à la consommation. Pour ces applications, les marqueurs génétiques conviennent tout à fait. Le marquage mécanique à petite échelle a également été utilisé à des fins de recherche. Sinon, la seule méthode de traçabilité possible demeure la tenue de documents de transport et l'étiquetage des emballages pour certifier l'origine des produits.
Dans la troisième partie, les auteurs décrivent les méthodes disponibles pour le traçage des crustacés. Ils passent en revue toute une gamme de procédés de marquage physique appliqués aux crustacés décapodes, comme l'injection de colorants biologiques (vert solide, bleu Niagara, trypan rouge et bleu) et les marques externes comme les rubans colorés, les fils métalliques et les marques à ancrage. De plus, ils examinent en détail un certain nombre de méthodes de codage interne, telles que les marques codées en micro-fils métalliques ou à base d'élastomère injecté. Comme pour les poissons, les techniques de génétique moléculaire sont également appliquées à l'étude des populations de crustacés et certaines d'entre elles sont décrites dans l'article. Les crevettes roses commercialisées sont le plus souvent conditionnées entières ou décortiquées après les indispensables opérations de tri, de lavage et de congélation, de sorte que le seul moyen d'en tracer l'origine est de la faire apparaître sur les documents de transport, les factures, les certificats sanitaires et l'étiquetage des emballages. Les conditions minimales d'étiquetage consistent en une description précise du contenu, à savoir, l'espèce, la quantité, l'identification du fabricant (nom et adresse), le lieu de conditionnement, l'importateur/exportateur ou le distributeur du produit, sans oublier le numéro du bordereau de chargement.

Mots-clés
Animaux aquatiques – Crustacés – Poissons – Marques – Marqueurs génétiques – Mollusques – Traçabilité.

■

Rastreabilidad de animales acuáticos

T. Håstein, B.J. Hill, F. Berthe & D.V. Lightner

Resumen
Resulta urgente disponer de métodos eficaces de rastreo para aplicarlos tanto a la investigación como a diversos tipos de operaciones propias de la acuicultura, así como al control del comercio de animales acuáticos y sus derivados. En lo que se refiere al marcado de peces, se han descrito muchos y muy diversos métodos. La aplicación de uno u otro dependerá del objetivo y la necesidad a que responda la operación. Para marcar a los moluscos y crustáceos, en cambio, existen contados procedimientos, debido sobre todo a dificultades prácticas.
Los autores describen en primer lugar los distintos métodos existentes para el rastreo de peces y productos de piscifactoría, por medio de: marcadores externos como los de Floy, los de Carlin o los transmisores pasivos integrados; el marcado químico con compuestos inorgánicos como nitrato de plata o de potasio,

pigmentos, oxitetraciclina, etc.; o diversos tipos de dispositivo electrónico en los que pueden almacenarse datos básicos como la cepa, la piscifactoría de origen o el peso de cada ejemplar. Una técnica que progresa con notable rapidez es el rastreo genético por tipificación del ácido desoxirribonucleico, aplicada a poblaciones salvajes o a bancos de reproductores criados en cautividad. Esta técnica ofrece un margen de fiabilidad muy alto a la hora de confirmar o refutar el supuesto origen, filiación o cepa de un animal o producto de origen animal, y puede utilizarse como prueba ante un tribunal.

En la segunda sección, los autores describen la rastreabilidad de moluscos vivos con fines de repoblación o de consumo humano. Los marcadores genéticos se han revelado muy útiles para tales menesteres. En la investigación también se ha utilizado el marcado mecánico a pequeña escala. Al margen de esos procedimientos, el único método de rastreo de moluscos vivos consiste en registrar en documentos específicos los desplazamientos de los animales o certificar el origen de los productos etiquetando su embalaje.

La tercera sección está dedicada a los métodos existentes para el rastreo de crustáceos vivos o muertos. Los autores describen un amplio repertorio de métodos para el marcado físico de los crustáceos decápodos, como la inyección de colorantes biológicos (verde sólido, azul celeste Niágara, rojo y azul tripán) o la aplicación de marcadores externos como cintas de colores, alambres o diversos marcadores que se sujetan con ancla. Por otra parte, los autores examinan en detalle distintos procedimientos de codificación interna, como el de micromarcadores codificados de alambre o el de elastómeros inyectados. Los autores describen asimismo algunas de las técnicas de genética molecular que se utilizan, al igual que en el caso de los peces, para el estudio de poblaciones de crustáceos. Los langostinos destinados al consumo humano suelen empaquetarse enteros o bien como colas a granel previamente seleccionadas, lavadas y congeladas, por lo que únicamente cabe determinar su origen mediante los documentos de transporte, las facturas, los certificados sanitarios y las etiquetas de los embalajes. El etiquetado debe indicar como mínimo el contenido del paquete, esto es, especie, cantidad, identidad del fabricante (nombre y dirección), lugar de embalaje, importador/exportador o vendedor del producto, además del número de certificado de embarque.

Palabras clave

Animales acuáticos – Crustáceos – Marcadores – Marcadores genéticos – Moluscos – Peces – Rastreabilidad.

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